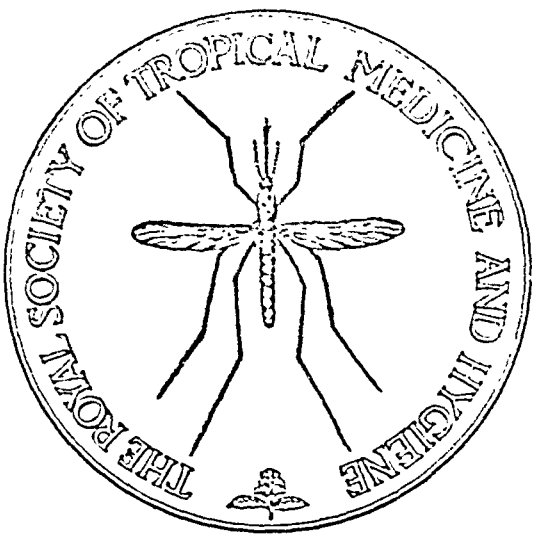
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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

PATRON - HIS MAJESTY THE KING.



ZONAE TORRIDAE TUTAMEN

VOL. XXXI. 1937-1938.

London :

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
MANSON HOUSE, 26, PORTLAND PLACE, LONDON, W.1.
Telephone: LANGHAM 2127. Telegrams: ANOPHELES, LONDON.

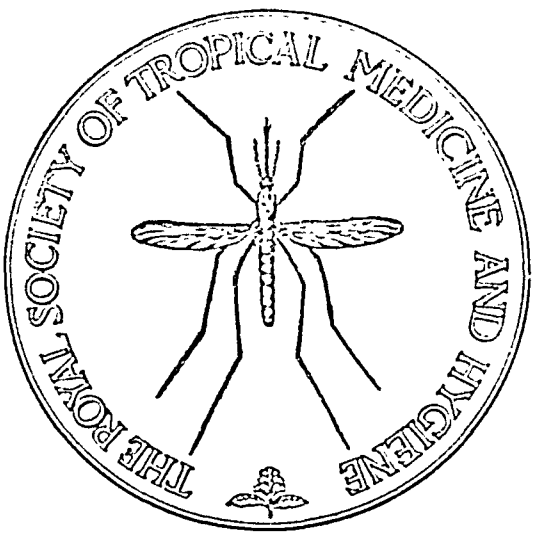
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Corrigendum.

Vol. 31, No. 5, p. 549 ("Investigation on the purification of water with respect to schistosome cercariae"):

By G. Witenberg, M.D., and J. Yofe, M.D.,

should read

By G. Witenberg, V.M.D., and J. Yofe, Doctor of Chemistry.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOL. XXXI. No. 1. JUNE, 1937.

Proceedings of a **Laboratory Meeting** held at the Royal Army Medical College, Millbank, London, S.W., at 8.15 p.m., Thursday, 18th March, 1937.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

DEMONSTRATIONS.

Hygiene Department of the Royal Army Medical College.

Modern methods of water purification in the field.

Col. A. C. Amy and Major J. S. K. Boyd.

Graphs and tables illustrating the effect of the new synthetic drugs on the incidence of malaria among British troops in India.

Dr. A. R. D. Adams.

1. *Entamoeba histolytica* infection of rabbit's stomach.

A heavy concentrated suspension of cysts was fed to the animal which died after 3 weeks with extensive amoebic ulceration of the stomach and of the small and large bowels.

2. *E. histolytica* infection of guineapig's colon.

A guineapig was similarly infected and developed extensive ulceration of the large bowel, and to a lesser extent of the small intestine.

3. Freshly penetrated miracidia of *Schistosoma haematobium* in *Bulinus (Pyrgophysa) forskali.*

Numerous viable ova from a Mauritian case of urinary schistosomiasis were placed in a Petri dish in water containing several snails. The miracidia emerging from these eggs vigorously attacked the snails. After about half an hour the snails were killed and fixed. Serial sections showed numerous freshly-penetrated miracidia.

4. Vermiform appendices invaded by ova of *S. haematobium.*

Routine section of all vermiform appendices removed at a Mauritian Government hospital disclosed the fact that many were infested with bilharzia ova. The majority of these cases had no history of either bilharziasis or of appendicitis.

5. *Onchocerca volvulus* larvae from the corneae and the skin of a European infected in Africa.

A European, aged 34, spent 4 years in Central and East Africa. On returning to England in 1934 he developed eye trouble and partially lost his sight. The cause of this was not determined until 1937 when larval *Onchocerca volvulus* were recovered from his skin and conjunctivae. He has had no nodules or skin lesions attributable to the infestation, and the only other indication of parasitization was an eosinophilia of about 40 per cent.

6. *Bertiella studeri* from man.

This parasite is normally one of primates. Ten cases of human infestation have now been recorded, and of these five have been found in Mauritius whence the present specimen comes.

Dr. W. E. Cooke.

Blood films from a case of mixed infection of *Plasmodium ovale* and *P. falciparum* from Zanzibar.

The patient, a missionary, age 23, was invalided to England for tubercular infection about a year after arriving in Zanzibar. On reaching England in February, 1937, he was found to be suffering from fever, enlargement of the spleen, and marked anaemia. Blood examination revealed the double malarial infection which responded rapidly to atebrin treatment.

Dr. N. Hamilton Fairley.

Tropical macrocytic anaemia in Macedonia.

Dr. FAIRLEY showed a series of blood films, bone marrow smears and Price-Jones curves illustrating types of anaemia encountered in Macedonia during some recent investigations there on blackwater fever. The most interesting case was one of tropical macrocytic anaemia.

The patient, a woman aged 25 years, had suffered from chronic malaria with splenomegaly and fever for many years and was 7 months pregnant. Five days after intensive quinine therapy commenced (2 grammes daily) she aborted. When examined 12 days later she was afebrile and profoundly anaemic, with a spleen extending to the umbilicus. R.B.C.s = 980,000 per c.mm.; W.B.C.s = 9,530 per c.mm.; neutrophils = 72·8 per cent.; lymphocytes = 21·6 per cent.; monocytes = 5·2 per cent.; eosinophils = 0·4 per cent. Pseudo-agglutination was marked and the sedimentation rate rapid. Van den Bergh—indirect reaction positive (2 units). The blood picture and the Price-Jones curve were typically megalocytic in type. Thus the mean diameter = 8·115μ, the standard deviation = 0·571μ, the coefficient of variability = 7·035 per cent. and the megalocytosis = 30·2 per cent. Marrow smears showed an intense megaloblastic response similar to that encountered in tropical sprue and pernicious anaemia. There were numerous primitive haemocytoblasts and many primary and secondary erythroblasts as well as normoblasts. True Ehrlich megaloblasts, however, with pink-staining cytoplasm were not common.

For 6 days the patient received daily injections of 8 c.c. of campolon. The reticulocyte response on the 7th day = 19·3 per cent. Within 19 days the R.B.C.s = 3,060,000 per c.mm.; this indicated a gain of 1,980,000 corpuscles per c.mm. or approximately 100,000 cells per day. The Price-Jones curve at this time showed restoration of the blood to a normal condition. The mean diameter = 7·016μ; the standard deviation = 0·5565μ; and the coefficient of variability = 7·931 per cent.

Findings similar to those cited above were observed in a number of other patients with malaria and blackwater fever in Salonika and will be published at a later date. The exhibits had a special interest since they demonstrated for the first time the existence of tropical macrocytic anaemia in Macedonia where the diets were often very deficient in sources of extrinsic factor. Marmite was not procurable in Salonika itself, but its adoption as a therapeutic agent even in hospital practice would be of the greatest benefit.

Dr. G. M. Findlay and Dr. R. D. Mackenzie.

Preparations showing lesions in monkeys, ferrets and other mammals infected with the virus of fowl pest.

The virus of fowl pest is ordinarily pathogenic only for fowls and a few other species of birds. As a result of continued intracerebral passage in mice both its neurotropism and its general pathogenicity are increased to such a degree that it then becomes infective for a large number of mammals and birds including the rhesus monkey, ferret, hedgehog, mouse, rat, field vole, duck and pigeon. The naked eye and histological lesions produced, more especially in monkeys and ferrets, bear a very close similarity to those seen in yellow fever and show that the virus of fowl pest must be included in a group that already comprises the viruses of yellow fever, dengue, Rift Valley fever and horse sickness.

Lt.-Col. S. P. James and Dr. P. Tate.

Preparations illustrating the recently discovered cycle of avian malaria parasites in reticulo-endothelial cells.

Photographs of some of the preparations shown are reproduced on a plate facing this page. Colonel JAMES, who was invited by the PRESIDENT to describe the exhibit, said it was concerned with the life cycle of the avian malaria parasite which Professor BRUMPT (1935 and 1936) found in domestic fowls in Ceylon in 1935 and to which he gave the name *Plasmodium gallinaceum*. This parasite, like other members of the family Plasmodiidae, has a schizogonic cycle of development in the circulating red blood corpuscles of the vertebrate host and a sporogonic cycle of development in the insect host which, as Professor BRUMPT showed, is the yellow fever mosquito *Stegomyia fasciata* (*Aëdes aegypti*). But it appeared from the work illustrated by the exhibit that it has, in addition, a hitherto unrecognized schizogonic cycle of development in monocytes and in reticulo-endothelial cells of the spleen, liver and kidneys; and particularly, in certain cases, in the reticulo-endothelial cells which line the capillaries of the brain. The first step in this finding was that in January this year Colonel JAMES observed in the cytoplasm of endothelial cells from the spleen and heart blood of a chicken which had died from the malarial infection, non-pigmented schizogonic phases of a parasite which bore little or no resemblance to the pigmented schizogonic stages of the malaria parasite as seen in red blood corpuscles. The phases that had been observed were shown to Dr. WENYON and were called, provisionally, "*x* bodies." Previously HUFF and BLOOM (1935) of the University of Chicago had found what seemed to be the same phases in various types of cells of the bone marrow of birds infected with *P. elongatum*; and RAFFAELE (1936) of the Marchiafava Institute for Malariology in Rome had found some of the phases in *P. elongatum* and also



FIG. 1.

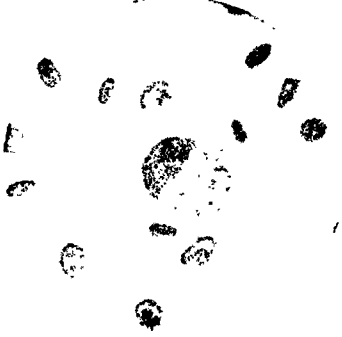


FIG. 2.



FIG. 3

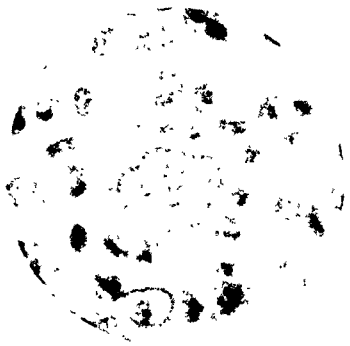


FIG. 4

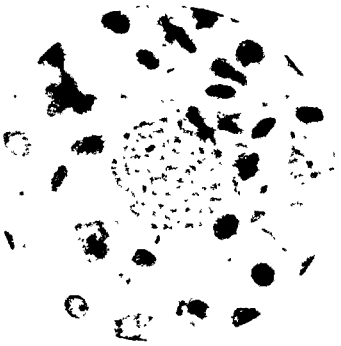


FIG. 5.

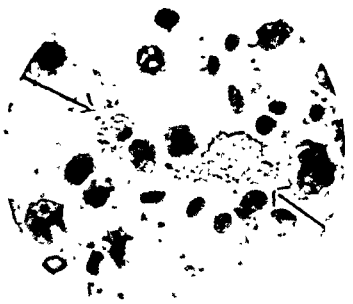


FIG. 6.



FIG. 7

PHOTOGRAPHS OF PREPARATIONS SHOWN BY COLONEL JAMES AND DR. TATE.

FIGS. 1, 2 and 3.—Schizonts of the endothelial cell cycle in cells from the heart blood of a fatal case.

FIGS. 4, 5 and 6.—Schizonts in smears of the spleen and liver. In 6 the arrow on the left points to the merozoites (about 30 in number) of a pigmented parasite which has "sporulated" in a red blood corpuscle, and the arrow on the right to the merozoites (numerous) of an umpigmented parasite which has "sporulated" in an endothelial cell.

FIG. 7.—A brain capillary distended and blocked by three large schizonts.

To face page 4.

in the reticulo-endothelial system of birds infected with *P. relictum*. Thus it appeared that observers working independently in different countries on three different species of avian plasmodia have found that at any rate some malaria parasites have an additional cycle of development in the vertebrate host which was previously unknown. As regards human malaria parasites, the occurrence of such a cycle in " tissue cells " instead of in red blood corpuscles was postulated by several investigators (JAMES, 1931) some years ago in an endeavour to explain why quinine is ineffective in preventing attacks of malaria due to the bites of mosquitoes and in preventing the relapses and recurrences which are such a troublesome feature of the human disease. In this connexion Colonel James and Dr. TATE repeated on chickens infected with *P. gallinaceum* some of the prophylactic and curative clinical trials with quinine and the synthetic anti-malarials which were made on other avian and on human plasmodia in 1931 and later years. The results seemed to them to indicate that the discovery of the endothelial cell cycle of the parasite opens up some interesting problems for chem-therapeutic research and may help to explain some of the clinical, therapeutic and epidemiological problems of malaria which have not yet been solved. Several of the preparations shown were from chickens which had been cured temporarily of the peripheral blood infection by the administration of two or more doses of quinine but had died later as a result of the development, in the cytoplasm of endothelial cells of blood vessels in the brain, of large schizonts which completely blocked the capillaries. (Fig. 7.)

Colonel JAMES remarked also that the discovery, apart from its probable bearing on clinical and therapeutic problems, raised a question of interest to systematists in that it did not seem correct any longer to define the Plasmodiidae as a family in which the whole of the vertebrate cycle of development occurs in the circulating red blood corpuscles. *P. gallinaceum* is evidently a parasite which, in the vertebrate host, has a cycle of development characteristic of members of the family Plasmodiidae ; but, in addition, it has a cycle of development resembling, in some respects, the schizogonic cycle in endothelial cells which is ordinarily described as being characteristic of members of the family Haemoproteidae. A preliminary note on these observations has already appeared in *Nature* (JAMES and TATE, 1937).

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———. (1936). *Ann. Parasit. Hum. et Comp.*, 14, 597-620.
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——— & TATE, P. (1937). *Nature*, 139, 545.
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Mr. H. S. Leeson.

Methods of recording sunlight at mosquito breeding places.

Two kinds of photometer were shown. The first is known as the " Eder-Hecht grey wedge photometer " and resembles a photographic printing frame of metal. Light passes through a milk-white glass and through another glass strip, which is transparent at one end but gradually increases in opacity towards the other end; then through a calibrated strip of celluloid. A print of the scale-lines is obtained on photographic paper during exposure. After toning, fixing and drying the paper the last transmitted scale line is read and the figure compared with the table of relative light values.

Regular exposures at the same time and place and for the same period each day (or alternatively, for the whole day) provide records of daily variation in total light reaching the place. Collections of mosquito larvae and light records from the same spot, when compared, will indicate if changes in light conditions affect the larval population. Accumulation of data over a considerable period is essential; this is only possible when the investigator is stationary.

Correction factors for the paper and for each part of the apparatus are obtained from the Observatory at Davos, Switzerland, from whence the apparatus originated.

The other instrument measures light intensity instantaneously. It was used during a recent visit to East Africa, where the bionomics of the different forms of *Anopheles* of the *funestus* series were being investigated. It consists of a photoelectric cell and galvanometer, the latter registering in milliamperes. At each exposure of the cell at mosquito breeding places it was also exposed to the open sky to measure the available light. The readings were then converted to percentages of available light. The tables and diagrams exhibited showed that larvae of *Anopheles funestus* were found in waters receiving not more than 70 per cent., the most favourable being those receiving about 20 per cent. *A. gambiae*, on the other hand, was commonest in totally unshaded waters.

The standardizing of this photoelectric photometer was very kindly done by Mr. P. ULLYOTT of Cambridge.

Photographs of the instrument in use and of some typical breeding places in East Africa were also shown.

Mr. C. Machattie.

Piroplasmosis in the hyaena.

The piroplasm was found in blood films made from the tip of the ear of a 20 months old hyaena (*Hyaena striata*) in Iraq. The organism which was present in the blood in fairly large numbers was of the *Nuttallia* type and very

closely resembled the parasite of the cat described by DAVIS* from the Sudan under the name of *Babesia canis*. In the blood films of the hyaena there occurred also a spirochaete of the relapsing fever type.

Dr. R. D. Mackenzie.

Naturally occurring spirillum in the vesicula seminalis of the mouse compared with the spirillum of rat-bite fever.

The seminal fluid of a number of " normal " white mice contains a spirillum closely resembling the causal agent of rat-bite fever (STROESCO)†.

Since attempts to passage this organism in either rats or guineapigs have been unsuccessful, and in view of the later observations of DUBOIS,‡ it would appear that this is a new and unnamed spirillum which should be differentiated from *Spirillum minus*.

The possible presence of this organism in stock mice should be remembered.

Dr. Manson-Bahr.

1. Blood films from a fatal case of malignant tertian malaria.

On the voyage from the Gold Coast, where he had just completed a 6 weeks' tour, the patient, an elderly but vigorous and healthy man, suffered from persistent severe diarrhoea and vomiting. There was no fever and malaria was not suspected. The day following his arrival in London he was admitted to hospital and was found to have a heavy infection of *P. falciparum*. In spite of intravenous quinine and other treatment he became comatose and died the following day with all the characteristic postmortem appearances of overwhelming malignant tertian malarial infection.

2. Blood films from a case of *P. ovale* infection from the Cameroons.

Dr. C. M. Wenyon.

Intestinal flagellates (*Trichomonas*, *Giardia*, *Hexamita*) of mouse in intestines of tsetse flies fed on mouse.

During the course of experiments made by Dr. J. C. BROOM and Major H. C. BROWN designed to infect tsetse flies with trypanosomes by allowing them to feed on the abdomen of infected mice, it was noted that occasionally

*DAVIS, L. J. (1929). *Trans. R. Soc. trop. Med. Hyg.*, 22, 523.
†STROESCO, G. (1936). *C.R. Soc. Biol.*, 121, 619.
‡DUBOIS, A. (1936). *Ibid*, 123, 1262.

in dissection shortly after feeding that little or no blood was present in the intestine, which was filled with a whitish fluid. One such fly dissected about three hours after feeding was found to have in its intestine diluted blood containing trypanosomes as well as other flagellates which proved to be those of the intestinal forms from the mouse. It was evident that when feeding on the abdomen of the mice the flies were liable to pass the proboscis through the abdominal and intestinal walls into the intestinal canal from which fluid and organisms could be imbibed.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL DISEASES AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

Proceedings
of an **Ordinary Meeting of the Society**, held at
Manson House, 26, Portland Place, London, W.1, at 8.15 p.m.,
on Thursday, 20th May, 1937.
Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

PAPER.

BEJEL: THE ENDEMIC SYPHILIS OF THE EUPHRATES ARAB.

BY

ELLIS H. HUDSON, M.D.*

There is doubtless general agreement that there exists among the lowly populations of large areas of the world a tremendous amount of disease due to *Treponema pallidum*, and it can hardly be denied that the character of syphilis in the indigenous population of Asia and Africa differs in many respects from European syphilis. This " indigenous syphilis " has been recognized in many places and given many descriptive names, but it has been little studied because of admixture and confusion in some places with venereal syphilis and in others with yaws. Bejel, the endemic syphilis of the Euphrates Arabs, is free from both sources of confusion, being propagated under biologically free conditions, untreated, and not linked with sex, and being found in a region far from the accepted geographical delimitations of yaws.

Geographically, the Arab world, consisting of Arabia, Mesopotamia, Syria, Palestine, Egypt, and the countries of Northern Africa, lies where it touches the three continents of the Old World—Asia, Africa and Europe.

* The author gratefully acknowledges the assistance of his wife, of his colleague Dr. SUSAN S. CROSLEY, and of Miss AGNES L. YOUNG and other members of the staff, in the collection of clinical data and preparation of the papers which form the basis of this report.

This clinical investigation was carried out while the writer was in charge of the clinic of the American Presbyterian Mission in Deir-ez-Zor, Syria, during the years 1924 to 1936.

Historically, the Arab race has had intimate contact with these three great population masses. Arab military power penetrated eastward to India and westward to the Atlantic and Spain ; commerce and war took Arab influence across the Mediterranean and over the bridge of Turkey into Europe. While the Arab trader and warrior was creating an Arab civilization reaching from the Straits of Singapore to the Pillars of Hercules, north into Europe and south into the heart of Africa, he was at the same time creating an Arab pathology out of the diverse disease elements which he carried with him and which he found in his new homes. This process of amalgamation and interchange is still at work in commerce and pilgrimage throughout the ramifications of the Mohammedan world.

Sociologically, the Arab race stands midway between the primitive rural life of the African bush and the highly organized urban life of Europe and America. Though alien blood has percolated into Arabia through slave trade with Africa across the Red Sea, and through commerce with India across the Gulf, with Persia through Mesopotamia, and with Turkey through Syria, the Bedouin of the Syrian Desert and the Peninsula is the native Arab. In his habitat of desert and goat hair tent he remains the primitive savage. Where he has abandoned nomad life and assumed the rôle of agriculturist and trader he has become a sociological hybrid. The cities which the Arab has created in the Near East reveal his hybrid nature.

The Arab world is therefore a " transition " world, historically, geographically and sociologically ; and since diseases change their character with change of " soil," one would expect to find in this area transitional forms of disease, reflecting the diverse geographical, historical and social currents of this region of the world.

The town of Deir-ez-Zor, situated in the Syrian Desert on the Middle Euphrates, on the 35th degree of latitude north, is a microcosm of this hybrid Arab world, and bejel, the endemic syphilis of the Euphrates Arab, exemplifies this intermediate quality, resembling venereal treponematosis of the Occident in some respects, and tropical treponematosis (yaws) in others.

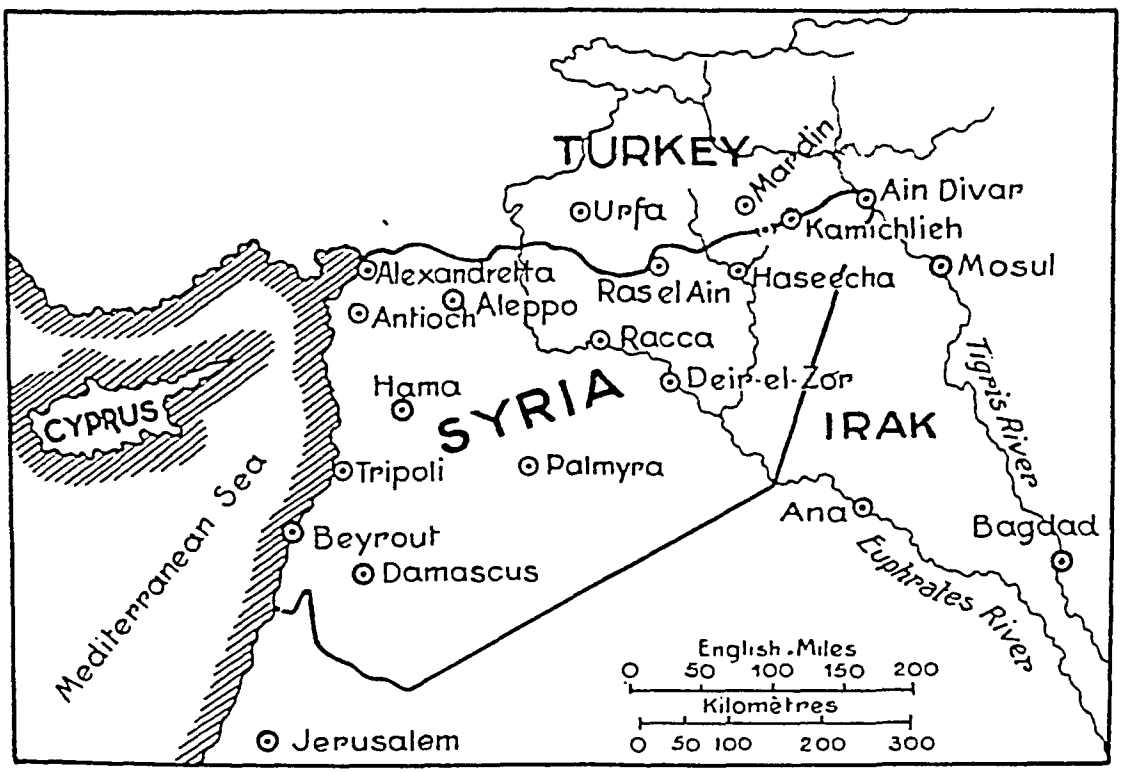
A glance at the map will remind one of the fertile crescent, beginning on the left with the Lebanon Mountains, running in a north-easterly curve along the south of Turkey, and ending to the east in Iraq. This well-watered crescent enfolds a semi-arid steppe known as the Syrian Desert across which—radially to the arc—run the Euphrates River and its tributary, the Khabur. The town of Deir-ez-Zor (Fig. 1, facing p. 16) lies near the junction of the two rivers at a point about the middle of the chord subtending the crescent. The straight-line length of the Euphrates from the Turkish to the Iraq borders is about 250 miles (400 km.), and the length of the Khabur is about 125 miles (200 km.).

The people who live along these two rivers are insulated from contacts with the outside world to a remarkable degree by the desert barrier, the nearest city being over 200 miles away, and most of them still live in the manner of their

ancient forbears. The settled population of the region numbers about 200,000, of whom 195,000 are indigenous Moslem Arabs. This number does not include the nomad Arabs of the Syrian Desert, whose position and numbers fluctuate with the seasons, and who are not included within the scope of this paper.

The Christian population of the region, numbering about 5,000, has little social significance and is not indigenous. The Christians are refugees and others whose homes are elsewhere ; they are to be found only in the towns.

The indigenous Moslems who comprise the bulk of the population are divided into two distinct groups, the townspeople and the Bedouins. The former number about 50,000 of whom 35,000 are to be found in Deir-ez-Zor, and the



remainder in other small towns. The Bedouins number 145,000, living in small mud villages strung along both banks of the Euphrates and Khabur. (Fig. 2.)

Venereal and Non-Venereal Syphilis.

Among the Christians the patronage of prostitution exists to a degree comparable to that in the West. Syphilis among them is known as *franghi* (the foreign disease), and the name carries with it the connotation of venereal origin and of shame. The Bedouins, on the other hand, call their syphilis *bejel* (Hudson, 1928), speak of it without shame, recognize it as a childhood disease, do not link it with sex, and resent the suggestion that it is the same

as the venereally acquired franghi. Christians never have bejel and Bedouins never have franghi.

Townspeople may have either—franghi if acquired venereally, and bejel if acquired non-venereally. The infants of town families are often sent out to the villages to be wet-nursed by Bedouin women, and they may thus contract bejel. In other instances bejel may appear among the children of a town family through some contact with a villager. On the whole, bejel is dying out of the towns, because barriers of treatment and segregation in the housed urban communities increasingly obstruct the normal routes of propagation among the children.

Though most of the town families sprang originally from Bedouin stock, townsmen and Bedouins are now sharply demarcated from each other by social distinctions of dress, language and mode of life. The economic status of the townsman—shopkeeper, usurer, artisan, professional man—is comparatively high, while that of the Bedouin, in constant struggle with the forces of nature, is just above the level of starvation. The townspeople despise the Bedouins as dirty and uncouth, and the dislike is mutual, so that there is little social or sexual intercourse between the two groups.

Prostitution is found only in the towns, and the Bedouins are not allowed in the brothels. The Bedouins live at a distance from the towns, which they can reach only on foot ; they have little money and little interest in the amusements of the town, and they do not touch alcohol ; their young men seldom enter military service, and their children do not attend the town schools.

In their own villages, the Bedouins have a strict sexual code which reduces promiscuity to a negligible quantity. The closely knit clan life still preserves the Arabs' highly developed sense of sexual propriety. Girls are married early, and the tribal organization sees to it that few women remain unmarried. The Bedouins do not tolerate sexual promiscuity, rape is severely punished, the marriage price is fixed by custom and paid to the bride's father, and polygamy as practised by the Arabs is a bulwark of public morals.

These are general considerations supporting the assumption that bejel, the syphilis of the Bedouins, is propagated non-venereally. Objective information on this point is furnished by the following clinical data (HUDSON, 1936b).

Of all the males, 15 years and older, admitted to the clinic in the course of 5,000 consecutive general new admissions, 40 per cent. were syphilitic. Of the Christian men in this series 12 per cent. were syphilitic ; of the townsmen 21 per cent. ; and of the Bedouin men 66 per cent.

In a consecutive series of 660 syphilitic men, 7·5 per cent. were Christians, 23·5 per cent. were townsmen, and 69 per cent. were Bedouins. In the same series, infection with syphilis was acknowledged by only half the Christians, by two-thirds of the townsmen, and by nine-tenths of the Bedouins. Two-thirds of the Bedouins acquired their syphilis in childhood. A history of repeated venereal exposure and of gonorrhoea was obtained only from Christians

and townsmen. Genital chancres were found only among Christians and townsmen. Syphilitic lesions of the Bedouins were mostly late manifestations of the disease, in the mouth, nose and throat, on the skin, and in the bones and joints. (Table I.)

TABLE I.

SYPHILITIC LESIONS AND HISTORY OF EXPOSURE TO VENEREAL INFECTION IN 660 SYPHILITIC MEN.

	Christians (franghi) 50	Townsmen (franghi or bejel) 156	Bedouins (bejel) 454
Syphilitic lesions:			
Ulcers of mouth, nose and throat	3	28	175
Ulcers and eruption of skin	4	18	152
Periostitis	1	4	45
Arthritis and effusions	0	3	15
Juxta-articular nodules	0	1	7
Plantar keratosis	0	0	7
Exostoses	0	0	2
Orchitis	2	5	8
Late penile ulcers	0	1	6
Chancres	21	15	0
Buboes	3	3	1
Tabes dorsalis	1	2	0
Hemiplegia	0	1	0
History:			
Habitual venereal exposure	12	12	0
Acute or chronic gonorrhoea	10	10	0

Many of the lesions in Christians and townsmen are linked with sex; those in Bedouins are late sequelae of their childhood infections.

Further evidence of the lack of sexual promiscuity among the Bedouins is furnished by the statistics of gonorrhoea among 7,000 consecutive new clinic patients. There were 149 cases of this disease, ninety-eight in men and fifty-one in women. Ninety-six of the ninety-eight men were Christians and townsmen; none was from the seminomad villagers, the remaining two being nomads of the eastern desert, one of whom was a soldier. Of the fifty-one women, all were either Christians or townswomen.

My colleague and I have never found gonorrhoeal cervicitis or pelvic inflammation in the semi-nomad Bedouin women, nor acute urethritis or its complications in the men.

In a case of venereal syphilis, the element of shame clouds the patient's anamnesis; the Bedouins on the contrary, readily acknowledge previous infection with bejel, and their statements have a high degree of accuracy, as shown by a

check with their reactions to the Kahn test. Table II summarizes the statements of 1,000 consecutive Bedouins of both sexes and all ages.

TABLE II.

BEDOUIN STATEMENTS AS TO TIME OF INFECTION WITH BEJEL (1,000 CASES).

Time of infection (age in years).	Men.	Women.	Children.	Total.
0 to 14	219	236	103	558
15 to 19	18	17	0	35
20 to 29	25	33	0	58
30 to 39	12	13	0	25
40 to 49	7	5	0	12
50 to 59	4	0	0	4
60 to 69	3	2	0	5
Uncertain	13	23	4	40
Infection denied—				
Kahn negative	30	33	25	88
Kahn positive	16	11	9	36
Not tested	13	24	55	92
Total	360	397	196	953
Blank records				47
			Total cases	1,000

60 per cent. of the adult Bedouins say they had bejel in childhood, and over 75 per cent. acknowledge the infection.

In the whole series of 1,000, including patients whose statements were lacking and children who had not yet had bejel, 55·8 per cent. had had bejel in childhood, and 13·9 per cent. named a later specific date of infection; 4 per cent. were uncertain and 21·6 per cent. stated they did not have the infection.

There were in the series 757 patients, male and female, 15 years and older, of whom 455 (60·1 per cent.) had had bejel in childhood; 139 (18·4 per cent.) others had had bejel at a later specific time, making a total of 594 (78·5 per cent.) who acknowledged syphilitic infection. Only 46 (6 per cent.) had had the initial infection after the age of 30.

In a series of 312 presumably healthy Bedouin non-patients, 175 (56·1 per cent.) had positive Kahn reactions of the blood.

The foregoing is evidence not only that bejel among the Bedouins is not a venereal disease, but that it has a community-wide distribution, and that it is usually acquired in childhood.

Clinical Course of Bejel (Hudson, 1933.)

At some time in early life, the Bedouin child contracts bejel from some other child in the acute stage of the disease. The spirochaete is usually passed from host to host by immediate non-sexual contact, and the transference is favoured by general uncleanliness, total lack of segregation and the succulence of the mucocutaneous lesions. Possible auxiliary factors in contagion are the use of a common drinking bowl, the habit of kissing and fondling, and the presence of the domestic fly, the louse and the flea.

Oral lesions are usually the first to appear, and the word bejel is used by the Arabs particularly for patches in the mouth. These patches desquamate but do not ulcerate. The saliva and the scrapings from the plaques swarm with spirochaetes of the morphology and characteristic motility of *T. pallidum*. About the same time papules appear about the genitalia and in the folds of the skin (Fig. 3) where there is warmth and moisture. Other papules may appear on the trunk and extremities (Figs. 4, 5), usually in circinate configurations. Spirochaetes are easily demonstrable in all these lesions. A roseolar eruption has been observed, but is rare: doubtless the pigment of the Bedouin skin sometimes causes it to be overlooked.

The process in the oral cavity may extend to the bones of the nose, producing softening and collapse of the nasal bridge. Less often there is tenderness and swelling of the epiphyses and periosteum of the long bones.

In the course of a year the early lesions disappear, leaving an apparently healthy child. Few of the Bedouin children ever receive any treatment; their parents regard the infection as inevitable and self-terminating.

It is said of the secondary lesions of yaws that they often leave permanent scars. This is certainly not true of the skin and mucous lesions of the early cases of bejel; these heal without leaving a trace. This difference may be due to a greater incidence of secondary invasion and tissue necrosis in the humid climates of yaws regions. The rainfall in Deir-ez-Zor is less than 5 inches per annum, the air is always dry, and suppuration is not common in superficial skin wounds of any kind.

Children who have not yet had bejel give a negative reaction to the Kahn and Wassermann tests, but their reactions become strongly positive as soon as they acquire the disease. The reactions remain positive for years—even throughout life—though the subject may present no further signs of the disease.

When an adult contracts bejel the mouth lesions usually appear first, followed by those of the skin and periosteum (Figs. 6, 7, 8); often these early lesions pass directly into the late forms without a marked latent period.

An adult who has escaped the infection in childhood is likely to contract it later from some child—often his own. It is obvious that it would be rare for a marriage to take place in which *both* partners had escaped prior infection. When this does occur, however, both parents may contract the disease from a

child, or one parent may get it and pass it on to the other, either by sexual or non-sexual contact. It is only this combination of conditions which would make possible the transmission of bejel through sex. In most marriages both parents have already had bejel ; where this is not so, it is generally true that one partner has had childhood bejel, and because of the lapse of time is not infectious to the other partner. Bejel is maritally acquired, therefore, in a very small proportion of cases, certainly in less than 1 per cent.

Illustrative Cases.

Case 5398. Male, aged 32, presenting a mucocutaneous eruption and a positive Kahn reaction. The patient and his wife and their 1 year old son all caught bejel at the same time from their 4 year old daughter, who had caught it from a neighbour's child.

Case 8387. Female, aged 28, presenting a mucocutaneous eruption ; the darkfield test was positive. Her elder son first had bejel, then her infant son aged 1 year, and then the patient herself.

At this point attention must be called to the fact that the initial or chancre stage is absent or, at any rate, cannot be recognized in bejel. Why this should be so is hard to explain, in view of the frequent finding of the chancre in venereal syphilis. One might suppose that the first lesion usually appeared in the mouth and was unrecognized, especially in the child, but this would not exclude the probability of finding an occasional chancre on the skin. The fact is that neither adults not children ever present initial skin lesions which might be considered chancres, and the Bedouins themselves never recognize such a beginning of the disease. Bejel is to them an *eruption*, first in the mouth and then on the skin. (Fig. 9.)

An explanation of this lack of the chancre stage may be found in the hypothesis of a different immunology in childhood-acquired syphilis. It is granted that the various tissues react allergically at different rates to the presence of the spirochaete. The common explanation of the two or three months' period intervening between the appearance of the chancre and the secondary eruption in venereal syphilis is that it takes that length of time for the skin to become " sensitive " to the parasite, and the maculopapular skin eruption is an expression of the development of hypersensitivity in that tissue. It may be that in untreated childhood-acquired syphilis there is already present—perhaps congenitally—such allergic hypersensitivity of the skin that the eruption appears immediately upon the heels of the primary invasion.

A fact that throws some doubt on this explanation is that yaws seems to have an initial or " mother " lesion ; yet it is also a largely untreated and childhood acquired treponematosis whose immunology one would expect to resemble that of bejel. Possibly this discrepancy may be due to the fact that the earliest yaws lesions always occur on the skin rather than the mucous membrane. Or it may be that observers of yaws—misled by the analogy to venereal syphilis—have been too prone to assign a chancre-like rôle to the earliest lesion of yaws,



FIG. 1.



FIG. 2.



FIG. 3.

FIG. 1.—Air view of Deir-ez-Zor and the Euphrates (*Reproduced by courtesy of L'Aviation militaire du Levant*). The river here splits into two channels, enclosing a long wooded island. The arches of the smaller bridge may be seen in the centre of the picture, and the suspension bridge to the right. The American Hospital is to the left of the latter. The villages of the semi-nomad Bedouins, and their narrow strips of irrigated land, line the banks of the river as it winds through the desert beyond.

FIG. 2.—The Bedouin villager's hut has a half-wall of mud, scraped up from the surrounding soil, and a roof of brushwood covered with grass. The hearth is in the centre of the hut, which is open on one side.

FIG. 3.—Case 7650. Child, aged 1 year; bejel of 1 month duration, probably acquired from mother, who had bejel in her childhood, and a large ulcer of pharynx and right tonsil two months prior to the child's infection. The child's eruption is mucocutaneous; darkfield positive. Note papulo-pustular eruption on the back, and condylomatous papules (and flies) on thighs and perineum.

To face page 16.



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.

FIG. 4.—Case 8122. Female, aged 4; mucocutaneous eruption, bejel, freshly acquired from neighbour child; darkfield positive. Note grouping of fine papules.

FIG. 5.—Case 8261. Male, aged 12; mucocutaneous eruption, bejel, duration 2 months, caught from neighbour boy. Round and arciform papules at nape and front of neck, folds of elbows, knees, groins and perineum. Patches on lips, tongue, pharynx and nasal septum. Kahn reaction and darkfield both positive.

FIG. 6.—Case 8816. Male, aged 20; bejel, 3 months duration, caught from neighbour child. Mucocutaneous eruption; on the skin, furfuraceous desquamation upon an erythematous base; severe itching. Kahn reaction and darkfield both positive.

FIG. 7.—Case 7004. Female, aged 30; bejel, 3 months duration; caught from neighbour children; husband had bejel in childhood. Mucocutaneous eruption; lesions on pharynx, tonsils, tongue, lips, neck, ante-cubital fossae, back, abdomen, genitalia, and popliteal spaces.



FIG. 8a.



FIG. 8b.



FIG. 9.



FIG. 10.



FIG. 11.



FIG. 12.

FIG. 8.—Case 7722. Male, aged 50; bejel, recently acquired from his own children; had not had it before. Patches appeared first in the mouth, followed by severe laryngitis, and then by universal skin eruption; shortly afterwards had periostitis of right ulna, metacarpals, both tibiae, and arthritis both knees. Kahn and Kolmer-Wassermann reactions positive; darkfield positive. Morphology of early skin lesions are figured (*a*) on the chest, (*b*) on the right arm.

FIG. 9.—Case 8741. The earliest bejel lesion observed. Male child, 1 year old; bejel, duration a few days, caught from mother who acquired it a month before. Child also had papule on tongue tip. Darkfield both lesions positive.

FIG. 10.—Case 5704. Male, aged 41; had bejel in childhood, followed in later years by recurring ulceration and extensive erosion of nose, hard and soft palate, pharynx and larynx.

FIG. 11.—Case 8795. Female, aged 8; had bejel 2 years before. Dacryocystitis, duration 1 month, pre-auricular and submaxillary adenitis; tibial periostitis. Kahn reaction positive.

FIG. 12.—Case 6460. Female, aged 28; had bejel in childhood. Ulcer at wrist, duration 5 months, 10 cm. diameter. Kahn reaction positive. Central healing with depigmentation; fungating margin.



FIG. 13.



FIG. 14.

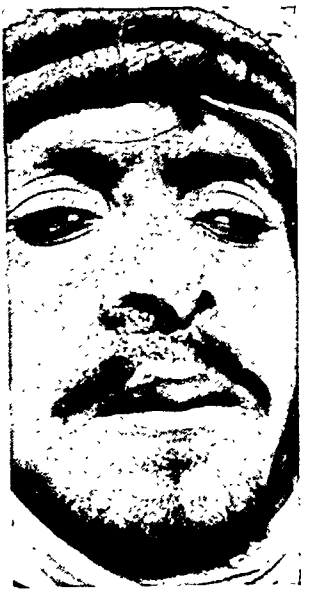


FIG. 15.



FIG. 16.



FIG. 17.



FIG. 18.

FIG. 13.—Case 8383. Male, aged 28; had bejel in childhood. Ulcerations right hip, of slow growth, 1½ years duration.

FIG. 14.—Case 6171. Female, aged 35; had bejel in childhood. Multiple ulcers both breasts, duration 6 months. Was bitten by nursing son who had bejel at the time. Tibial periostitis. Kahn reaction positive.

FIG. 15.—Case 8711. Male, aged 28; caught bejel from brother's children 3 years before, a universal eruption which disappeared without treatment. Papule appeared at right nostril 1 year before, subsequently extending downward on lip, and inward into nose; stippled surface under crust. Kahn reaction positive.

FIG. 16.—Case 3234. Male, aged 14; had bejel in early childhood, followed by ulceration of both tibiae, and erosion of soft palate, both tonsils and pharynx. Kahn reaction positive. Note forward bowing of tibiae.

FIG. 17.—Case 4357. Female, aged 6; had mucocutaneous eruption of bejel at the age of 3. Itching and fissuring lesions of palms, arms, soles and legs appeared at the age of 5.

FIG. 18.—Case 8828. Male, aged 56; had bejel in childhood. Circumscribed areas of scalp, loss of hair and pigment, duration 2 months. No signs or symptoms of neurosyphilis. Kahn reaction positive.

instead of grouping it with the manifestations of allergic hypersensitivity. It is admitted by many that in yaws the initial lesion is often absent or unidentifiable, and it is apparent that "chancre immunity" as seen in venereally acquired syphilis is absent in both bejel and yaws.

The late lesions are the next consideration in the clinical course of bejel. The most common of these is ulceration of the soft tissues at the back of the mouth, involving soft palate, tonsils, and pharynx (HUDSON, 1937b). The ulcer begins as a small erythematous patch which rapidly breaks down, and enlarges with the production of a yellow tenacious slough. Deglutition is very painful. This condition if untreated lasts for months, but finally heals spontaneously, with the production of sheets and bands of scar tissue, which may include all the tissues contiguous to the pharynx. Sometimes the process extends downward to and into the larynx, with the production of hoarseness and stridor, and when healing eventually takes place the cicatrix may actually occlude the larynx, necessitating tracheotomy to preserve life.

These ulcerations equal in number all other late lesions combined, and are of high diagnostic value, whether active or already healed. They frequently produce changes in the quality of the voice. Unfortunately they cannot be photographed. A very coarse maize, eaten in the form of a loaf only partially cooked, forms the staple—and usually the sole—food of the Bedouins. It may be that irritation caused by this rough diet is responsible for the frequency of these late ulcers of the throat.

Ulcerations in the throat may extend to the bones of the nose, or lesions may develop independently in the hard palate or the septum. In either case a mass appears within the nose, often erodes through the hard palate into the mouth, and breaks down bony tissue in all directions. The degree and character of mutilation resembles that of gangosa, or rhinopharyngitis mutilans. (Fig. 10.) Grey areas resembling leucoplakia are often to be found on the inner surfaces of the lips and cheeks.

Syphilitic dacryocystitis is not rare (Fig. 11), and affections of the nasal and malar bones may produce thickenings at the base of the nose suggestive of goundou, though a fully developed goundou lesion has never been observed.

The bulk of the remaining late lesions occur in the skin and bones, and trauma often determines their position. A very common site is the hip, due to the fact that the Arab sits on the floor, resting either on one hip or the other. Ulcers often develop at the site of cautery burns.

The unit of the skin lesion is the papule, which soon becomes multiple, spreading in fan shape. Ulceration may follow, with slow healing on one side and extension on the other. Sometimes secondary invasion creates deep ulceration, but the converse is probably more common—the heaping up of granulation tissue in a fungating mass which exudes serum and becomes covered with a thick crust. (Figs. 12, 13, 14, 15.) When the crust is lifted a stippled surface of moist granulation tissue is exposed.. The dryness of the air discourages

suppuration and probably prevents a greater degree of mutilation. The bareness of the landscape and the lack of underbrush reduce the likelihood of scratches and wounds of the extremities such as are found in primitive people living in more humid climates. Late skin lesions of traumatic origin situated on the lower extremities are, therefore, not so common as might be expected.

Trauma often determines the site of the bone lesions and probably accounts for the frequency of periostitis of the tibial crests. Headache is a common symptom, and is often worse at night. General bone pains, described as throbbing in character, are usually present in bejel, both early and late, and are doubtless due to a general periostitis. In the long bones this sometimes becomes a periosteo-ostitis, with formation of ulcers which eventually heal with excess callous formation and the production of bony spurs and sabre tibiae. (Fig. 16.)

General adenopathy is characteristic of bejel. In the early cases all the superficial glands are enlarged and individual glands may be prominent and soft, though suppuration does not take place. In the late cases the epitrochlear and cervical chains are always palpable, hard, shotty. The inguinal and other chains are also usually palpable.

A feature of interest in bejel is the presence of juxta-articular nodules (HUDSON, 1935). This pathological condition—so rare in venereal and treated syphilis—is not rare among the Bedouins, five having been found in an unselected consecutive series of 236 adult males, an incidence of 2·1 per cent. These were found at the usual sites proximal to joints and on bony crests, and one was observed on the costal grill. They respond quickly to antisyphilitic therapy and biopsy shows the usual histopathology.

Hyperkeratosis of the plantar surfaces is common among the Bedouins, doubtless due to trauma and epithelial overgrowth on the soles of the feet of syphilitic subjects compelled to walk barefoot winter and summer (HUDSON, 1936a). The condition is manifested in two forms ; either as foci of cornification on the soles of the feet at the points that bear the weight of the body, or as a diffuse thickening of the whole skin, which tends to form fissures in cold, wet weather, and to present a rough moth-eaten surface in dry weather. Ringworm-like lesions of the palms and soles are also sometimes seen. (Fig. 17.)

Patchy depigmentation of the skin appears not rarely among the semi-nomads, either in the extensive scars of the skin lesions of bejel, or elsewhere in areas unrelated to the syphilitic lesions (HUDSON, 1936a). The discoloration often appears about the hands and the feet, but may involve the whole body, producing a piebald effect. It is impossible to detect any change in the texture of the skin in the decoloured areas. The patches grow by slow extension, but the border is quite undifferentiated from the remaining area.

Alopecia, or patchy baldness, with or without loss of hair pigment in the same area, may be found in late bejel cases (Fig. 18).

Hypertension is virtually unknown in Bedouin patients and cardiovascular lesions practically absent. (HUDSON and YOUNG, 1931). In a series of over

1,000 adult patients of various ages, the systolic pressure of only seventeen was over 150 mm. of mercury, and in all our experience with bejel my colleague and I have never found a clinically substantiated case of aortic valve disease or aneurism.

Clinical evidence of cerebrospinal syphilis in bejel patients is extremely small. In the course of a large series of tests the number of abnormal pupillary reactions, abnormal upright station (Romberg), and abnormal tendon reflexes was exceedingly small. Among 5,000 general clinic admissions syphilitic disease of the nervous system was diagnosed in six Christians and two townsmen of the better class. All of these gave a history of venereal infection and presented the usual late pathology of venereal syphilis. Five relatively inconclusive cases of neurosyphilis were found among the Bedouins who had had bejel, though they far outnumbered the Christians, and exhibited nine times their syphilitic morbidity.

In a series of thirteen bejel patients, twelve of whom were males, all of whom had strongly positive standard Kahn reactions of the blood, and most of whom had either early or late active and open lesions of bejel, the cerebrospinal fluid Kahn reaction was weakly positive in three, doubtfully positive in two, and negative in eight. The sugar was increased in one, the globulin in two, the cell count averaged something over 10 per c.mm., and the pupils, knee jerks and Romberg tests were normal.

The conclusion to be drawn from these facts is that the syphilitic process extends to the nervous system in bejel, as it probably does to all other organs of the body. Increases in sugar, globulin, and cells are indications of irritation, and the occasional positive Kahn reaction in the cerebrospinal fluid is further evidence of the presence of the disease. But the damage done by the spirochaete in the nervous system does not apparently extend to the point where clinical symptoms of neurosyphilis are produced.

Influence of Bejel upon the Second Generation.

It is generally recognized that venereal syphilis may affect human propagation at a number of points, viz., lessened fertility, increase of miscarriage, stillbirth and neonatal death, and the production of the dystrophies of the congenitally (prenatally) acquired infection. On the other hand, it has not been known whether similar deleterious effects are produced upon the second generation by non-venereal syphilis acquired in childhood. Bejel gives some information upon this point (Hudson and Crosley, 1936a), as shown in Table III, summarizing 2,500 parental reports.

This table compares the three social groups, the Christians with a morbidity of 10 per cent. venereal syphilis, the townspeople with a syphilitic morbidity of 35 per cent., mostly non-venereal, and the Bedouins with a morbidity of 90 per cent. non-venereal syphilis. It is immediately apparent that there is no

TABLE III.

SUMMARY OF PARENTAL REPORTS.

Reports of	Number of individuals.	Total living children.	Average number living.	Total dead children.	Average number dead.	Total miscarriages.	Total pregnancies.	Percentage miscarried.	Number reporting no miscarriages.	Percentage reporting no miscarriages.
Christians:										
Fathers	133	346	2·6	212	1·6	54	612	8·8	77	58
Mothers	383	821	2·1	748	2·0	249	1,818	13·7	207	54
Totals	**516**	**1,167**	**2·3**	**960**	**1·9**	**303**	**2,430**	**12·5**	**284**	**55**
Townspeople:										
Fathers	362	1,039	2·9	815	2·3	213	2,067	10·3	174	48
Mothers	662	1,775	2·7	1,386	2·1	513	3,674	14·0	352	53
Totals	**1,024**	**2,814**	**2·7**	**2,201**	**2·1**	**726**	**5,741**	**12·6**	**526**	**51**
Bedouins:										
Fathers	429	1,179	2·8	959	2·2	217	2,355	9·2	241	56
Mothers	648	1,630	2·5	1,562	2·4	360	3,552	10·1	397	61
Totals	**1,077**	**2,809**	**2·6**	**2,521**	**2·3**	**577**	**5,907**	**9·8**	**638**	**59**

Fertility and child mortality are slightly greater in the Bedouins, and the incidence of miscarriage is lower; yet the syphilitic morbidity of the Bedouins is three times that of the townspeople and nine times that of the Christians.

marked difference between the three groups in spite of their great disparity in syphilitic morbidity. Fertility and child mortality are slightly greater in the Bedouins, and the incidence of miscarriage is definitely lower. The percentage of Bedouins reporting no miscarriage at all is higher than that of the other two groups.

These are gross figures and include miscarriages and childhood deaths due to a multitude of causes. But if non-venereal syphilis were a causative factor of moment, it would certainly increase the miscarriage frequency rate and the childhood mortality rate in the townspeople who present three times the syphilitic incidence of the Christians, and in the Bedouins who present eight or nine times that of the Christians.

As a matter of fact, the showing of the Bedouin community is even better than appears in the table. For the Christian cases of syphilis are usually treated, those among the townspeople are often treated, but those of the Bedouin community rarely receive any treatment. In the second place, the health hazards run by the Bedouin child are much greater than those of the Christian child, and considerably greater than those of the town child. Again, the Bedouin women work hard throughout pregnancy, and are subjected to conditions of life much more conducive to miscarriage than those of the Christian or townswomen.

It appears, therefore, that the Bedouin women, in spite of much less favourable environment and much heavier syphilitic morbidity, are more fertile, produce as many living children, and are less apt to miscarry, than the townswomen and the Christians, with their better housing, much higher economic level and access to treatment. One must conclude that a high syphilitic incidence does not necessarily produce a weak and degenerate stock, and that even a 90 per cent. morbidity of non-venereal syphilis is compatible with a high degree of racial virility and fertility.

As to congenital or prenatal transmission of bejel, it is probable that the Bedouin child is usually born free of the disease, though both parents have had bejel previously and present positive Kahn reactions at the time of reproduction. The Bedouin child who has not yet had bejel does not have the facies or present the dystrophies of congenital syphilis.

Infants and young children do not present the manifestations of bejel until they are handled and fondled by older children who have open lesions of the disease. Kahn reactions are always negative before and positive after infection with bejel. Bejel invades a family and strikes children of widely varying age at the same time; its course may be traced from child to child through such a group. Instances are not rare of parents infected from their own children; an " early " infection may appear in an individual quite advanced in age, and even in a grandparent.

It is a recognized fact that the children of congenitally syphilitic parents are healthy in an overwhelming majority of cases. The evidence of bejel

supports the view that the same is true of the children of parents who have acquired their syphilitic infection in childhood.

It is apparent that syphilis, when propagated under the epidemiological conditions characteristic of yaws, acts like yaws in sparing the second generation. Each generation, therefore, faces its own bejel infection, as each generation faces its own yaws.

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As to the laboratory diagnosis of bejel, data have been secured by the use of the darkfield* (Hudson, 1936d), the standard Kahn reaction (Hudson, 1932; Hudson and Young, 1933), the presumptive Kahn reaction (Hudson, 1936c), and the Kolmer-Wassermann reaction (Hudson, 1937a). Spirochaetes indistinguishable from *T. pallidum* were uniformly found with the darkfield apparatus in the lesions of fresh bejel infections. They were also found, but with more difficulty and less constantly, in the late lesions, and it was assumed that such late positive cases were illustrations of the phenomenon of relapse, an assumption which agreed with the clinical conditions.

The standard Kahn reaction in the Deir-ez-Zor laboratory always gave about 60 per cent. positive reactions in any given Bedouin adult series, and this was true whether the series consisted of patients or of presumably healthy non-patients. In a series of Bedouin sera which were sent to the laboratory of the American Hospital in Beirut, the standard Kahn positive percentage was the same. That laboratory also kindly carried out the Kolmer-Wassermann test on the same sera, and though there were 11·5 per cent. anti-complementary reactions, the reaction was positive in 45 per cent. of the series.

In a carefully controlled analysis of 290 individuals, in whom the standard Kahn was negative or doubtful in all but eighteen, and in whom the presumptive Kahn reaction was positive in 130, it was shown that this more sensitive test revealed the presence of latent syphilis in a further proportion of the Bedouin community, estimated to be in the neighbourhood of 15 per cent. Adding this 15 per cent. to the 60 per cent. revealed by the standard Kahn reaction makes a serologically positive percentage of 75 in the Bedouin community.

This agrees roughly with the proportion of 75 per cent. who state that they have had bejel. If one eliminates the infants and children who have not yet had bejel, and adds the individuals who present syphilitic scars in spite of negative blood reactions and negative or uncertain histories, the proportion of syphilitic cases in adult Bedouins rises to over 90 per cent.

Analysis of these various series shows that no adjustment of the percentages need be made to rule out the possible presence of falsely positive reactions due to conditions other than syphilis, such as leprosy, malignant neoplastic disease, jaundice, pyrexia, pregnancy, malaria, or tuberculosis.

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Treatment of Bejel. (Hudson and Crosley, 1936b.)

From the standpoint of treatment the Bedouin subject presents peculiar problems. In the first place, he often seems indifferent to the lesions of bejel. Most children are allowed to go through the early stage without treatment, and adults carry mutilating ulcerations for long periods apparently without complaint. Secondly, the Bedouin seminomad soon becomes restive under the conventions of the town and may make off for home at any time without warning. Thirdly, he often abandons treatment if he does not immediately improve, or he infers from slight improvement that he is cured. Fourthly, the Arab has a hazy conception of all time intervals longer than the day and will not return regularly for treatment at intervals greater than one day. Finally, engaged in a constant struggle against starvation, he cannot afford to pay much for treatment or remain long away from work.

It is obvious that it is impossible to treat the bejel patient along the lines of modern antisyphilitic therapy. It is questionable, however, in view of the character of bejel, whether such treatment is necessary or even desirable.

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The situation demands one or more preparations of bismuth which are cheap, easily prepared, always ready for use, and not affected by exposure to heat and air; preparations which can be given in daily injections; which will give a perceptible improvement within 1 or 2 days; which will be slowly enough absorbed to prolong the bismuth effect for some time after treatment is over; and which will not give undesirable side-effects in nephritis and stomatitis.

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Almost without exception the patients under this régime show subjective and objective improvement within one or two days, due to the spirillicidal activity of the quickly absorbed tartrate, and they continue to improve after discharge, as they slowly absorb bismuth from the salicylate deposits.

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Of 1,000 bejel patients treated in this fashion, receiving an average of 75 cg. of the metal, the lesions of 28 per cent. were completely healed, and a further 49 per cent. were improved. The result was unknown in 13 per cent. and 10 per cent. were not changed.

The short intensive course of bismuth seldom reverses the positive Kahn reaction, and often makes it stronger. Occasionally, patients healed with bismuth have recurrences of bejel lesions. This not unexpected finding presents no difficulty, as healing is again secured with more bismuth.

The success of this type of treatment must be judged in the light of its objective. If the objective is control of infectiousness, then it is obviously effective, for it heals early, late, and recurrent lesions. If pursued for a period over a given area of population it must reduce greatly the incidence of bejel, merely by healing open lesions and preventing the formation of new cases. The short intensive course of bismuth is especially effective against mucocutaneous eruptions, and ulcers of the pharynx. These are the most common early and late lesions, respectively, and the lesions from which the spirochaetes are disseminated in greatest abundance.

The records show that the average length of time spent under treatment, and the average number of injections submitted to, increased steadily throughout the 4 years' trial of this treatment, a testimony on the part of the Bedouin community to the acceptability and efficacy of this schedule. It should be noted that attendance at the clinic was purely voluntary, and no treatment was given entirely gratis.

The Arabs themselves try to control the early lesions of bejel with the use of blue copperas, touching the sores with copper sulphate crystals in mediaeval fashion. They have also used mercury inhalations from time immemorial, and found it of great benefit in spite of the fact that the teeth sometimes loosen and fall out subsequently. But mercury, even with modern methods, is not suitable because the margin between its therapeutic and its toxic doses is too narrow.

The fact that the arsphenamines should be given at intervals of several days, in uninterrupted and unabbreviated series, makes them also unsuitable ; for the Bedouin patient will not and cannot give the necessary co-operation. When they are used, the patient receives one or more series of subcurative doses which are progressively ineffective, unsound pharmacologically, and not without risk to the patient.

Bismuth is a more active spirillicide than mercury and has a much wider margin between therapeutic and toxic dosage. It is a weaker spirillicide than arsenic in the form of arsphenamine, but it has the advantage of prolonged effect, and the power of stimulating the natural defensive mechanisms of the body. Bismuth is more suitable for the capricious primitive patient, because if he abandons treatment early—as he often does—he has not been harmed, nor has the success of possible later treatment been prejudiced. This is not true of treatment with the arsenicals.

In the mass treatment of bejel, the childhood reservoir of the disease should be attacked by searching out and treating all fresh cases; and sporadic cases of relapse or gummatous ulceration in late cases should be constantly sought for and brought quickly under treatment. Working from these two angles, the infection which is already present can be "sealed up," and the disease eventually eliminated by prevention of new cases in the child population.

It should be the aim of medical administrators to carry out the mass treatment of primitive peoples with the least dislocation of native life and usages. Experience with the short course of bismuth in the treatment of bejel shows that by this method the disease can be wiped out without changing in any respect the social and moral sanctions of the Arab village communities.

For mass treatment of native peoples of all ages—many of them children—under unavoidable conditions of heat, insects, and general uncleanliness, intramuscular injections of bismuth are obviously suitable, on grounds of simplicity of administration, low toxicity and clinical effectiveness; and they have the further real advantage of very low cost.

Four points in regard to bejel merit recapitulation: (1) That bejel is a treponematosis is established by the character of its early and late lesions, the quality of latency, the uniform presence of a spirochaete indistinguishable from *T. pallidum*, the positive precipitation and complement fixation reactions, and the favourable response to antispirochaetal drugs. (2) Bejel is acquired in childhood or early in adult life. (3) It is a non-venereal disease, not linked with sexual intercourse. (4) It is found in both sexes and all ages, and to the same degree in the sick and in the presumably well components of the population; it is so common in the seminomad Bedouin communities as to be well-nigh universal.

The Significance of Bejel.

Four features of significance emerge from a consideration of bejel:—

(1) *The Relation of "Bejel" to Disease Nomenclature.*

If the present writer is correct in assuming that the aetiological agent of bejel is *T. pallidum*, and if it is granted that all pathological manifestations of *T. pallidum* are to be called syphilis, then it follows that bejel is syphilis. But bejel is so different from venereally acquired syphilis in epidemiology, clinical course, and apparent pathology, that there is justification for the introduction of the native name of the disease into the literature. This avoids the cumbersome and inadequate qualifying and descriptive terms which have been used elsewhere, such as native, indigenous, primitive, rural, tropical, colonial, untreated, childhood-acquired, extragenital, non-venereal, mucocutaneous, endemic and

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pandemic. There is precedent for the introduction of a native word : in the 18th century the African word " yaws " was used to designate the tropical treponematosis of that region.

A major contribution which bejel makes to the knowledge of syphilis is its delineation for the first time of syphilis biologically unrestrained, and free from the venereal factor. There is undoubtedly a great amount of the bejel type of syphilis in the world, and fragmentary references to it have appeared in the medical literature from time to time ; but only on the Euphrates has it been possible thus far to study the activities of the spirochaete free from venereal epidemiology, and in an area far from the endemic regions of yaws. Bejel is therefore the prototype of non-venereal syphilis, a clinical entity, susceptible of study and worthy of a distinctive appellation. The word " bejel " is introduced into medical literature not to confuse, but to clarify thought in regard to spirochaetal disease.

(2) *The Relation of Bejel to Yaws and Syphilis.*

Bejel is of significance in the discussion as to the identity or duality of the treponematous diseases, yaws and syphilis. In the first and third columns of the accompanying differential table a well-known textbook is quoted verbatim (MANSON-BAHR, 1935). Bejel has been inserted in the second column, and it is immediately apparent that it occupies an intermediate position between the other two diseases, with possibly a tendency to resemble yaws rather than syphilis.

Bejel resembles yaws in non-venereal childhood-acquisition, community-wide dissemination, high diagnostic value of the patient's own statement regarding infection, absence of congenital transmission, morphology of the skin lesions, relative escape of the eyes, the cardiovascular system, the central nervous system, and the viscera, the lack of constitutional disturbance, the lack of infantile dystrophies, the failure to impair fertility and virility, and the presence of gangosa, juxta-articular nodules, and patchy depigmentations. Both bejel and yaws tend to disappear rapidly when brought into contact with civilizing influences. In both, the objective of treatment and the problems of treatment are the same; and, in both, bismuth is the drug of choice.

On the other hand, bejel resembles syphilis in its constant involvement of the mucous membrane in the early stage, in the occasional finding of alopecia, in many of its general pathological aspects, and in its extra-tropical geographical location. In general, there is no clear-cut differential either between bejel and yaws, or between bejel and syphilis.

LACAPÈRE (1923) has described the Arab syphilis of Morocco, showing how greatly it differs from western syphilis ; BLACKLOCK (1932) has pointed out that the descriptions of yaws in Ceylon and the West Indies do not fully correspond ; WILSON (1934) states that the yaws he has seen in Panama is much less virulent than that which he has seen in Haiti ; RAMSAY (1925) observed that the

DIFFERENTIAL TABLE.

Yaws.*	Bejel.	Syphilis.*
Not congenital	Resembles yaws	Congenital.
Primary sore—		
Extragenital	Resembles yaws	Usually genital.
Secondary stage—		
(*a*) Typical yaws pathognomonic; furfuraceous desquamation and plantar lesions characteristic	(*a*) Frequently resembles yaws	(*a*) Seldom imitates framboesia.
(*b*) Mucous membranes not affected	(*b*) Mucous membranes always affected	(*b*) Mucous membranes affected.
(*c*) Itching common	(*c*) Resembles yaws	(*c*) Itching rare.
(*d*) Alopecia unknown	(*d*) Resembles syphilis	(*d*) Alopecia may occur.
(*e*) Eyes unaffected	(*e*) Resembles yaws	(*e*) Iritis common: choroiditis and retinitis rare.
Tertiary stage—		
(*a*) Visceral lesions absent	(*a*) Resembles yaws	(*a*) Visceral lesions occur, *i.e.*, pericellular cirrhosis, gumma of liver, kidney, etc.
(*b*) Nervous system not usually affected	(*b*) Resembles yaws	(*b*) Nervous system prone to infection; tabes, G.P.I.
(*c*) C.S. fluid always negative Wassermann	(*c*) C.S. fluid often positive Wassermann	(*c*) C.S. fluid usually positive Wassermann.
(*d*) Blood vessels: no endothelial proliferation as in syphilis	(*d*) Probably resembles yaws	(*d*) Endarteritis obliterans of viscera-cerebral thrombosis.
Yaws better resisted. Constitutional disturbance slight; great exuberance of eruption and cheloid scarring	Certainly resembles yaws	Syphilis attacks constitution, affecting the vital structures.
Does not respond to mercury	Responds to mercury	Responds well to mercury
.....		
Gangosa, juxta-articular nodules, and patchy depigmentations not rare	Resembles yaws	All three rare.

*The columns headed Yaws and Syphilis are taken from *Manson's Tropical Diseases*; but the present writer is responsible for the additional symptoms referred to below the dotted lines, and for the whole of the centre column (Bejel).

yaws lesions of natives in the hills differed from those of the same people when dwelling on the plains. There are doubtless many environmental factors involved, such as native practices, native diet, atmospheric humidity, rainfall, mean temperature, elevation, insects, amount of clothing, degree of treatment. None of these epidemiological and ecological factors has yet been evaluated in relation to treponematosis, and throughout the world there are all variations of these " unknowns," producing an infinite number of combinations.

One gets the impression, therefore, that differences between the various forms of treponematosis may be quantitative rather than qualitative, of degree rather than of kind ; that these differences might be explained on epidemiological grounds if the influence of all environmental factors were given full value. One might tentatively construct a spectrum with venereal syphilis at one pole, followed by Moroccan syphilis, then bejel in an intermediate position, followed by the more severe yaws types, and ending with yaws seen in its mildest form.

But the pathology of bejel has not been studied ; the pathology of yaws is confused in many respects ; and we do not know certainly how far syphilitic pathology can be modified by changed epidemiological conditions. Ecto- and meso-dermotropisms have no place in the differentiation of syphilis and yaws if bejel is due to *T. pallidum*, for the treponema of bejel is apparently as ecto-dermotropic as the treponema of yaws.

At the present stage of knowledge it is equally rational to take either of two views. One may emphasize the similarity of bejel to venereal syphilis, and conclude that no matter how closely epidemiological conditions may approximate syphilis to yaws, syphilis still remains syphilis and does not cross the dividing line into yaws. Or one may emphasize the similarity of bejel to yaws, and conclude that when the epidemiological conditions of syphilis approach those of yaws, the character of the resulting disease is such that it cannot reasonably be distinguished from yaws.

The issue may be stated : Is there a specific biological difference between the treponemata of syphilis and yaws, or can their differences be explained on the basis of their respective epidemiologies ? Bejel does not offer crucial evidence in favour of either view, but it does make a major contribution to the discussion to this extent, that it demonstrates conclusively that the more syphilis resembles yaws in epidemiology, the more it resembles yaws clinically. Some will accept this as *conclusive* evidence of biological identity. Others—among whom is the present writer—will consider bejel a strong argument for the unity of the two diseases, but will continue for the present to consider the question not proven, pending further study particularly in the fields of epidemiology, pathology and animal experimentation.

I shall, therefore, for the present continue to maintain the position I adopted in this speculative field 9 years ago in describing bejel as a spirochaetal disease due to *T. pallidum*, a variant of the syphilis-yaws treponematosis.

(3) *The Relation of Bejel to the History of Syphilis.*

Bejel is significant from the standpoint of medical history. As it exists to-day in the Euphrates Arab, it has the biological character of an ancient disease. The Arab and the spirochaete live in equilibrium with each other, the host-parasite relationship is balanced, so that the life of the host is not cut short, nor his progeny blasted prenatally. Whether this is due to attenuation of the treponema through numerous free passages from host to host without any intervention of treatment, or whether it is the result of an acquired racial resistance, or whether it is due to a combination of both factors, it is impossible to say. In any case, the result could only be secured in the course of many generations, even centuries. There is every reason to assume that bejel is an ancient disease of the Bedouins, and no reason to suppose that it is a disease of recent origin.

Beyond this point, speculation on the origin of bejel takes one of two directions. If one assumes that treponematous disease of man originated in humid tropical jungles, then bejel may be an intermediate stage in the evolution of the disease in its march north to become the syphilis of the civilized world. There has always been commerce and slave trading from central Africa and Abyssinia across the Red Sea into Arabia. One would then assume that bejel has been modified toward the " syphilis pole " by climatic and other epidemiological conditions.

On the other hand, if one assumes the American origin of syphilis, then bejel may have gravitated through centuries under the influence of social and climatic conditions from a venereal to a childhood disease, greatly resembling yaws. Either assumption depends for its force upon the power of environmental factors to make profound changes in the clinical character of a disease. But whatever responsibility for European syphilis may finally be laid upon the shoulders of Columbus's sailors, the present writer finds it difficult to see how they could have had anything to do with the introduction of bejel to the Euphrates.

The medical historian, puzzled by the absence in ancient medical literature of any clear reference to a disease corresponding to modern syphilis, is inclined to believe that the disease must have been absent. But the ancient world was predominantly rural ; the majority of its inhabitants probably lived under conditions comparable to those of the Euphrates Arab to-day. It may be, therefore, that the Bedouins are presenting a contemporary picture of syphilis as it existed in ancient times—non-venereal, acquired in childhood, untreated, and disseminated throughout the community. Periods of great sexual licence would then be possible without carrying the dissemination of syphilis in their train. Syphilis would then be lost in the confusion of childhood exanthemata, and the later manifestations—unrelated to the early infection, and more or less self-limited—would be lumped with sore throats, skin ulcers and rheumatic bone pains.

If to-day a medical historian were to examine a modern Euphrates village, his search for venereal histories and lesions, and for the visceral pathology which he associates with venereal syphilis, would be unrewarded, though 75 per cent. of its inhabitants have positive serological reactions and say they have had the disease, and surveys show that 90 per cent. of the adults are syphilitic. A search for a condition resembling bejel might profitably be made in the ancient medical literature of childhood disease.

(4) *Bejel the Archetype of Syphilis.*

Lastly, bejel is of significance in a consideration of the biological interaction between *T. pallidum* and the human race. It is apparent that in bejel the treponema is allowed to go its own way, unrestrained by human intervention. It is, therefore, the original and pure form of the disease. To achieve success as a parasite the spirochaete must employ every possible route to get itself transferred from one human body to another. In a dirty, careless, poorly-clad, closely huddled and untreated population, the facilities for making this transfer are innumerable, especially among the children. Syphilis under these circumstances is a childhood infection, and this fact undoubtedly affects the immunological course of the disease.

A disease passed freely through many generations of children will not only develop a peculiar immunology, but will eventually develop a peculiar pathology, tending always to a greater degree of mildness, and striking less and less at the vital organs of the host. Something akin to domestication takes place in the parasite ; there is no opportunity for a violent swing in the host-parasite relationship which would lead to crippling or death of the host ; there is no intruding factor to " step-up " the virulence of the parasite. The host responds generously to the invasion of the parasite, with a florid eruption which bespeaks a pre-existing receptive immunological state, and it has long been recognized that the skin is a fertile source of antibodies.

The host suppresses the spirochaete in the course of a year, and parasite and host exist in apparent harmony for years—perhaps for life. Only when trauma, or some other accidental factor subsequently intrudes does the disease manifest itself again. Each time this occurs, there is a renewal of the parasite-tissue struggle, which may waver in favour of one or the other for a while, but eventually ends in healing and the production of a scar. Scarring indicates that the host has again prevailed and redressed the balance.

When the spirochaete has unlimited opportunities to get across from body to body it is a childhood disease. But civilized man has narrowed down the field of opportunity by creating barrier after barrier—shoes and clothes, soap and water, segregation of the family within the house, isolation and treatment of the sick. In fact, in civilized life the spirochaete with rare exceptions has been cut off from all but one avenue of sufficiently close bodily contact to allow transfer.

Syphilis has, therefore, lost its primordial character of childhood disease

and has become a venereal disease. Its venereal character is not the result of some mechanism or tropism in the syphilis germ itself, but of the restraining circumstances—the epidemiology—which civilization has thrown about its activities. Venereal syphilis is, therefore, an artefact erected by civilization upon the base of the childhood bejel type of syphilis. It is a specialized type of syphilis, the outgrowth of a special and more or less artificial set of environmental factors.

In the epidemiology of venereal syphilis there enter several factors which modify first its immunology and then its pathology. In the first place, instead of being acquired in early life it enters the individual when the organs are already formed; the immunological response of a fully matured body is different, the physical and mental stresses and strains and the intercurrent infections of mature life enter the equation. In venereal syphilis the female acquires the disease at the period of reproductive activity, and congenital transmission, infection of the foetus, miscarriage and stillbirth are made possible. The visceral pathology and the likelihood of prenatal infection stem directly, therefore, from the epidemiological fact of venereal transmission of the spirochaete. Treatment in civilized life also comes in to change the course of the disease, inadequate or unsystematic treatment being especially productive of disastrous results. The infection in a civilized country reaches only a small percentage of the population; the spirochaete does not tend to become attenuated, nor is there opportunity for the development of a racial immunity. In fact, the net result of the impact of civilization upon syphilis is to step up the virulence of the disease.

The following question has been raised by several writers: Is it wise to clear up yaws in a native people when the chances are that the much more serious venereal syphilis will be introduced in its place?

In the opinion of the present writer, the question is beside the point. If a certain primitive people were taught to live in civilized fashion, they would lose their yaws anyway, and their syphilis would be venereal. If the people were left unchanged in their primitive habits, but cleansed of their yaws, and if venereal syphilis were subsequently introduced, the venereal syphilis would in the course of time assume the relatively benign characters of bejel.

SUMMARY.

1. The settled population of the Middle Euphrates valley comprises 5,000 Christians, 50,000 townspeople, and 145,000 seminomad Bedouin villagers. A desert barrier effectually isolated this region in the past, and communication with the outside world is still difficult. Social and economic barriers separate the three groups from each other.

2. Venereal practices and diseases are found only among Christians and townspeople. That they are not found among Bedouins is due to economic and social rather than moral factors.

3. Syphilis is the most common disease of the region, and it appears in two forms, *franghi* which is venereally acquired, and *bejel* which is acquired in childhood. Franghi—comparable in all respects to western syphilis—is found among Christians and townspeople, bejel among townspeople and Bedouins.

Seventy-five per cent. of the adult Bedouin villagers say they have had bejel, and from a consideration of the histories, the positive serological reactions, and the syphilitic lesions and scars, it is permissible to conclude that over 90 per cent. of the adult Bedouins are syphilitic.

4. Bejel is highly contagious and is usually contracted by and from children. It is an afebrile malady with little effect upon the general constitution. It exhibits mucocutaneous and periosteal lesions in the early cases, which may persist for a year; after a latent period which may last for many years, late lesions appear in most individuals, in the form of mucocutaneous relapse or gummata of the skin and the nasal and long bones. Juxta-articular nodules, plantar hyperkeratoses, depigmentations, and alopecia may be found in late cases. The nervous and cardiovascular systems and the viscera escape serious damage.

5. The children of parents who have had bejel—like the children of congenitally syphilitic parents—usually escape prenatal infection, and the dystrophies of congenital syphilis are absent. Bejel does not decrease fertility nor increase the rate of miscarriage; the child mortality rate is not increased.

6. Spirochaetes of the characteristic morphology and motility of *Treponema pallidum* are found constantly and abundantly in the lesions of freshly acquired bejel, and in the late cases of mucocutaneous relapse.

Bejel is uniformly accompanied by positive precipitation and complement fixation reactions of the blood; 75 per cent. of adult Bedouins have positive serological reactions.

7. Bejel as it exists in the childhood reservoir is usually untreated, and passes without restraint from child to child or child to adult. It is, therefore, a biologically free disease, unrestricted by any barrier, unmodified by any treatment, and favoured by general crowding and uncleanliness.

Cure of the individual bejel patient is not a practical objective. The therapeutic objective in this endemic syphilis should be not individual cure, but prevention of new cases in the community. This can be done by healing the open lesions—abolishing the infectiousness—of the cases already present. This objective can be secured with bismuth alone at low cost, without risk to the patient, and in a time schedule adapted to the temperament of the Arab. Bismuth is less toxic and more effective than mercury; it is more suitable to the conditions of bejel than the arsenicals, and also easier of administration, less hazardous and much cheaper.

8. There is undoubtedly much syphilis of the bejel type in the world, but it has often been confused with venereally acquired syphilis or with yaws. Purely non-venereal, acquired in childhood and free from admixture with either

venereal syphilis or yaws, bejel is a clinical entity meriting the retention of its distinctive name.

9. Bejel occupies in many respects an intermediate position between syphilis and yaws, and demonstrates that the more syphilis resembles yaws in epidemiology, the more it resembles yaws clinically. At the present stage of knowledge bejel offers suggestive but not conclusive evidence in favour of the identity of syphilis and yaws.

10. Bejel has the character of an ancient disease; the two hypotheses of its origin are considered. It may be that syphilis of ancient times was like modern bejel, in which case the search for references to venereal syphilis in ancient medical literature is beside the point. References to a disease resembling bejel might be found among the ancient descriptions of childhood exanthemata.

11. The view is advanced that bejel is the archetype of syphilis. It is suggested that the similar immunology and pathology of bejel and yaws stem from their non-venereal epidemiology. It may be that the urban portion of the human race, by imposing a venereal epidemiology upon the spirochaete, has changed the clinical course and the pathology of syphilis, producing in civilized countries a far more serious disease than the original syphilis of rural children.

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Discussion.

Prof. D. B. Blacklock: I should first like, if I may be permitted to do so, to congratulate Dr. Hudson on his very prolonged labours on this subject, and also on the very able exposition of it which he has given us to-night.

Some years ago, in West Africa, I began to harbour, in secret, some rather heretical doubts as to the infallibility of the data and the criteria by which we were to diagnose yaws from syphilis; those misgivings I committed to paper and sent to a journal, and the editors were unfeeling enough to publish them. The result was a chorus of protests by authorities on yaws and syphilis from various parts of the world. There were first those who said there were two diseases; they ranged from people who said they had only to glance at a case to see which disease it was, to people who said they were not sure how they did it but they felt convinced they could see some difference between the two diseases. In the next category were arrayed those who said there was only one disease; they said this question had been settled long ago, that they had known it for years and, they asked, what is anybody butting in for at this time of day and flogging this dead horse?

In my efforts to criticize the accepted criteria I put first the following: *Yaws not congenital, syphilis congenital.* The suggestion was made that it was hardly wise to compare yaws, a disease of childhood, with syphilis as a whole, and especially that it might be fallacious to compare yaws with venereal syphilis. I had no figures of childhood syphilis, and so I was compelled to use congenital cases. Dr. Hudson wrote to me and said he was working in an area where there was apparently an abundance of child syphilis, and ever since then I have followed the progress of his work with great interest. You have heard Dr. Hudson to-night produce his case clearly, and I think it shows firstly, that this syphilis in children—bejel—is not transmitted congenitally to the next generation and, secondly, that syphilis cannot be distinguished from yaws simply on account of the genital site of the primary lesion in syphilis. I think that those are the two main pillars which support the structure of the differential diagnosis between yaws and syphilis, and those who have heard Dr. Hudson to-night will agree that he has shown how inadequate those pillars are to support any such structure as has been built upon them.

There is one particularly interesting question which he has gone into to-night. I suggested that it might be dangerous in view of the new methods of rapid treatment of yaws to proceed without being certain about some particular points: that there were two eventualities worth considering. The first was, if you gave to a yaws patient a non-radical treatment, that is, one which will

alleviate the symptoms and get rid of the eruption and the infectiousness, you might leave the person liable, later on, to get neuro-sequelae. The second eventuality was this: If you cure a person who has got yaws, will you not then leave him in such a position that when he gets older he can acquire adult venereal infection? I gather from Dr. HUDSON that he does not consider that this latter question is to be viewed with much apprehension. The experience of COOK in Uganda, which I mentioned in my paper was also slightly reassuring on this point. But I hope that Dr. HUDSON or some speaker in the discussion will tell us from his experience whether there is any real danger of the half-treated cases showing neuro-sequelae later in their lives, a matter to be taken note of from the public health point of view. From the public health aspect it is fallacious, when you are dealing with bejel or yaws questions, to talk of the individual as if he were different from the community, because the aggregate of the infected individuals in any such area is practically all the population: in the bejel area, for example, some 90 per cent. Whatever, therefore, you do to the individual in such places you are doing to a very large part of the population. I ask again, do we run the risk of having neuro-effects as a result of partial treatment?

There are figures in certain colonial reports which attract attention. For instance, in the report for Kenya in 1935, in respect of a large mental hospital, out of 120 consecutive cases 22 per cent. showed neuro-spirochaetal effects. Dr. PATERSON, Director of Medical Services, discusses this incidence and says it may be due to the organisms of yaws or of syphilis, and enquires whether it is the aftermath of the yaws epidemic which is dying down or the first result of that increase of venereal syphilis which is now being noted? Another interesting report is that of Jamaica, where there is a lunatic asylum in which institution there are housed 2,000 patients. Of 201 patients admitted during the year and examined with regard to Kahn and Wassermann reactions, thirty-four were found to have a positive reaction; and of fifty-nine patients already in, five were positive. Of 229 people there who died during the year, thirty-three were general paralytics. The Medical Superintendent, Dr. HEWSON, is not overstating the case when he writes that the persistent annual increase of insanity is a matter of grave concern.

From the wider public health point of view, the position there is also particularly interesting, especially when you look at the finances of the institution. In Jamaica, of the total expenditure in the Colony, 8·3 per cent. is on behalf of the medical department, that amounts to £190,000; of that sum nearly £42,000 is spent on this lunatic asylum; that is to say, from one-fifth to one-fourth of the total medical expenditure for the Colony is on this one institution. Therefore, from the public health point of view it is important for us to know the origin of those neuroses which occur in association with spirochaetal disease. One would like to know such things as this: are they the result of a childhood infection with

an extragenital sore, or are they the result of an adult venereal
Have they had any treatment before they were admitted as patien
what drugs were used, and how much treatment have they had
one would like to know whether or not the numbers of those who ha
treatment before admission are in excess of those who have
treatment ?

Answers to these questions would prove very valuable from the pu
point of view. They are all questions which are raised by the paper

In conclusion, I would say how interested I have been in foll
HUDSON'S work and hearing him to-night ; and I am sure you will all
me when I say that he has added very substantially to our knowl
extremely difficult subject.

Col. L. W. Harrison said he was struck with the similarit
Dr. HUDSON'S experience and that of others like Dr. MACQUEEN in
who had encountered a practically identical condition, which was
the *British Journal of Venereal Diseases* for January, 1934. Dr.
mentioned in his bibliography the earlier work in Dalmatia, Croti
Herzegovina and Asia Minor on the same type of disease about whic
been hearing to-night. He could remember reviewing a paper by
worker* in Abyssinia, who told much the same story and stated
Kaffir race south of Gore inoculated their children early in life int
so that by the time they were married they would be well over thes
He was interested in comparing Dr. HUDSON'S plan for preventing
of these diseases, by using bismuth injections, with that suggeste
MACQUEEN. Dr. MACQUEEN thought that the best procedure was to
plan suggested by OPPENHEIM for treating congenital syphilis, n
administer orally the pentavalent arsenical preparation known va
stovarsol, kharophen, orarsan, or spirocide which was first intended t
by the mouth as a prophylactic against syphilis. OPPENHEIM sugg
it would be good for treatment of congenital syphilis, and it has be
adopted for that purpose. MACQUEEN thought it advisable to ster
people temporarily by establishing a dispenser in an infected village to
that all infected persons took the drug. His plan was to give each ad
two tablets of 0·25 gramme on the first day, and three on each of the ne
then a rest of 3 days was given, and the treatment was continued
patient had received as many tablets as he weighed in kilogrammes ;
patients were treated with smaller doses on the same lines.

In neuro-syphilis, he considered that a distinction should be drawn
syphilis affecting the supporting structures of the central nervous sy

*NÄGELSBACH, E. (1926). *Arch. f. Schiffs- u. Tropen-Hyg.*, 30, 121.

syphilis affecting the parenchyma of the brain. The term neuro-syphilis is often used for what is actually vascular syphilis. Many of those cases about which they had heard that night showed evidences of changes in the cerebrospinal fluid, but that might indicate only that the supporting structures of the central nervous system were affected, a condition similar to that found amongst the Arabs of Northern Africa and the Negroes of North America who suffer less from neuro-syphilis than do white people there.

He agreed with Professor BLACKLOCK that a great amount of work had yet to be done on the subject of the relationship of inadequate treatment to the late development of neuro-syphilis, but he would repeat the hope that when this work was done those engaged in it would distinguish between the two kinds of syphilis in the central nervous system that he had mentioned. Some work had already been carried out; for example KEMP, of Chicago, concluded that inadequate treatment did not promote the incidence of neuro-syphilis. He knew that inadequate treatment with arseno-benzol preparations alone would promote the development of meningo-vascular syphilis, but he did not think that inadequate mixed treatment led to neuro-syphilis. Since 1916, there must have been thousands of people in this country who had had inadequate treatment, judging by the number who desert from the treatment centres; and yet general paralysis of the insane is on the decline. He would suggest that the absence of miscarriage in the cases described by Dr. HUDSON was due to their having been infected in childhood. Although any syphilitic woman may transmit the disease to the foetus, the tendency certainly does become less as the infection grows older. He would suggest that in many of the cases there was a chancre on the tonsil or in the mouth and he would like to ask Dr. HUDSON if he had noticed any unilateral enlargement of the sub-maxillary or the submental glands in the cases he saw, because unilateral enlargement of these glands would suggest a primary syphilitic lesion in the mouth.

Dr. H. S. Stannus said he wished that Dr. HUDSON had not included in his paper the table of differential diagnosis; it had already been attacked by Professor BLACKLOCK in relation to yaws and syphilis and now it seemed to him to be open to criticism on the question of bejel. It was true that Dr. HUDSON had realized the danger for he said that when he used the word " resembles " he did not imply that there was any " identity."

Dr. HUDSON identified bejel with syphilis but at the same time assigned to it a place intermediate between that disease and yaws, giving his reasons for so doing.

Although he thought that Dr. HUDSON'S observations formed a most valuable addition to the knowledge of clinical syphilis, he was very doubtful if it was fair to argue that bejel was a link in a syphilis-yaws chain.

(*a*) The fact that bejel was contracted commonly in childhood, non-venereally, was merely an epidemiological fact and proved no resemblance to yaws.

(*b*) It should be noted that in the table the primary sore in yaws was said to be extragenital and " bejel resembles yaws " ; whereas, of course, the initial lesion in yaws *may* be genital, and in bejel as Dr. HUDSON said elsewhere, a primary lesion is never seen. Syphilis should never be thought of as a purely venereal disease when according to STOKES in nearly 20 per cent. males and 36 per cent. females the primary chancre is extragenital. Incidentally, it should be noted that the terms venereal syphilis and non-venereal syphilis were improperly used when it was thereby suggested that the disease in the two cases necessarily followed a different course. One should speak rather of venereally acquired syphilis and non-venereally acquired syphilis.

It was interesting to note that bejel contracted by an adult by sexual intercourse remained bejel just as yaws similarly contracted remained yaws.

Dr. HUDSON stated that the skin eruption of bejel frequently resembled yaws in contradistinction to syphilis. The word *resemble* was one which constantly led to trouble in medicine. What one wanted to know was : Is the skin eruption ever *identical* with that in yaws ? After having read all Dr. HUDSON'S descriptions of the disease and seen his photographs, the speaker was convinced that the skin lesions of bejel do not resemble those of yaws.

Dr. HUDSON suggested that the primary lesion in yaws was often absent or unidentifiable. He did not think this was the experience of those familiar with the disease. It would probably be more correct to say that on the occasion when the patient was seen the primary yaw might be unidentifiable.

The fact that the mucous membranes of the mouth and naso-pharynx were *always* affected in bejel appeared to be most significant in its relation to syphilis.

In regard to yaws—after going through all the literature—he remained unconvinced that lesions of mucous membranes ever occurred apart from " spreads " across muco-cutaneous junctions or direct inoculation. The adeno-pathy in bejel was general : in yaws localized ; the alopecia in bejel was as in syphilis : it did not occur in yaws. The positive Wassermann reaction given by the cerebrospinal fluid was important.

Dr. HUDSON said that pathologically bejel probably *resembled* yaws ! He would like to know whether this was so or not. Furthermore, Dr. HUDSON had stated that bejel " *certainly resembled yaws* " in being " better resisted : constitutional disturbance slight : great exuberance of eruption and cheloid scarring "—as opposed to syphilis. He ventured to think that this was hardly a true picture. It should be remembered, however, that many cases of syphilis were first diagnosed late in life at a skin clinic from some dermal manifestation

without there having been any previous symptoms, while, on the other hand, the " picture of misery " presented by large groups of yaws cases was well-known to many of those present. As regards keloid formation, this appeared to be a racial peculiarity and was in no way due to yaws specifically.

Dr. HUDSON suggested that yaws in one country differed from yaws in another country and that for this reason yaws, bejel, native African syphilis and European syphilis could be considered as forming a series. With this view he could not agree. The disease syphilis, including bejel, varied in its clinical picture but there always appeared to be distinguishing characters separating it from yaws. Though in yaws there might be variations in the clinical picture there appeared to be no difficulty, if each case was taken as a whole, in reaching a correct diagnosis. While gangosa was extremely common in association with yaws in Oceania it was said to be unknown in Indo-China. The great difference in incidence of juxta-articular nodules has also been noted, but the secondary eruption of yaws considered in detail still appeared to be characteristic.

In regard to the pigmentary changes in the skin, he thought it was important to distinguish between lack of pigmentation, which might result from scarring following any lesion of the skin associated with loss of substance, and the other characteristic depigmentary changes which might occur in syphilis and yaws. That, characteristically seen in yaws, was a patchy depigmentation, often associated with some hyperkeratosis, occurring on the hands and feet, wrist and ankles. In one of Dr. HUDSON's photographs a part of an extended hand was seen showing a condition like this. On the other hand, in another photograph a patient was shown exhibiting large leucodermic patches all over the body. That condition he did not think was ever due to yaws and he would doubt if it were actually due to bejel. Idiopathic leucoderma was common among dark-skinned races.

Dr. HUDSON had written " A search for a condition resembling bejel might profitably be made in the ancient medical literature of childhood disease " ; he (HUDSON) thought that descriptions of what he believes to be the archetype of syphilis—bejel—would in this way be discovered.

He would suggest that it was not necessary to go so far back. The description given by GILCHRIST of a " very infectious distemper prevailing in many places " (Scotland) called *sibbens* or *sivvens* appeared to refer to a disease having a very close resemblance to bejel of which they had heard this evening.

That " the soil " upon which syphilis was grown plays an important part, as Dr. HUDSON suggested, was probably very true ; the " chemical make-up " of a race, endocrine and vitamin balance might all play a part. Even the older writers believed that scurvy might alter the aspect of syphilis. This was an aspect of syphilis among native races which might well repay research.

Dr. H. M. Hanschell said that this was not the first time he had had the pleasure of listening to Professor BLACKLOCK on the subject of yaws and syphilis, and each time he heard him he became the more convinced that yaws was not syphilis. It seemed to be assumed that bejel and syphilis were the same disease, caused by the same species of parasite. But on listening to Dr. HUDSON, it was evident that there were such grave differences as to make one reluctant to believe that they could be the same species. There was the matter of the primary chancre which Dr. HUDSON had failed to observe. With much diffidence, he disagreed with Colonel HARRISON; where an infection falls on 95 per cent. of the children he could not believe that in the main it was due to a tonsillar infection; it was more likely that these folk were continually rubbing in each others' infection than receiving it by droplet means. Dr. HUDSON had failed to observe a primary lesion, except in one case, a photograph of which he had shown: this was an erosive papule on a child's back. He had had the fortune to see syphilitic lesions of the skin of a varied assortment of races of mankind, many with deeply-pigmented skin, and at no time had the primary lesion looked like a papulo-erosive chancre. Wherever it had occurred on the outer skin, it had always the same character, well described as a "raw beef" chancre, distinct in appearance from the papulo-erosive chancre which was confined strictly to the sulcus or precoronal sulcus and the under surface of the foreskin just as it leaves the sulcus. Elsewhere, it always had the "raw beef" character, not at all like that which had been shown to-night as the only example of an early lesion Dr. HUDSON could produce. That part of the skin which comes into relation with the outside world might show lesions varying in appearance according to the environment. The fact that these people live in a very dry climate might account for the character of the lesion, just as, we know well, a trauma of the skin might determine the site of a lesion, so also might the kind of climate determine the character of a lesion.

But there still remained the fact that the spirochaete of bejel did not penetrate the choroid and infect the parenchyma of brain or spinal cord. And that was a distinct difference, because the coloured races which acquire the primary chancre of syphilis do suffer from parenchymatous infection of the central nervous system, even if not to the same extent as the European. At the Seamen's Hospital they had had patients from East Africa, Somaliland and India showing parenchymatous syphilis of the central nervous system with the characteristic changes in the cerebrospinal fluid. There was thus no racial immunity. Again the spirochaete of bejel seemed unable to pass the placenta to infect the foetus, another very striking difference. One was forced to conclude that it was a different spirochaete.

Finally, he would refer to the plea that one should hesitate before offering these unfortunate people the benefits of treatment because this might mean that later in life they would acquire genital syphilis or neuro-syphilis. That reminded him of the very strong objections he had heard expressed in 1912, to the use of salvarsan because cases were reported in which the cure of a chancre with salvarsan had been followed by the contraction of another. He thought that this plea not to treat bejel because these things might happen afterwards was rationalization, using that word in—as Professor BLACKLOCK would agree—a scurrilous sense; it was merely an excuse to avoid the difficulty and expense of giving these people the treatment which we could give them, which one is quite sure would alleviate this suffering; and which they ought to have.

Dr. P. Manson-Bahr said that he thought that the spirochaete of syphilis and yaws were originally the same organism but that like other living things, various types had been evolved as a result of local conditions leading to different methods of infection and other characteristics. He mentioned the typhus group of diseases of which there are now many varieties though it seemed to him that originally there had been but one. If this was the case, there was no end to the discussions as to the identity or otherwise of the various types.

Dr. C. C. Chesterman said he would join issue with Dr. STANNUS and tell him that all those lesions which they had seen illustrated on the screen spoke to him of yaws rather than of syphilis. He had had experience of 30,000 cases in 15 years, and he had seen in yaws patients practically every lesion which Dr. HUDSON had shown; the only two lesions he had not seen were alopecia and mucous membrane involvement and that might be accounted for by the varying humidity of the climate. In an arid country a plant must put its roots further down, and some allergic factor or biological necessity of the spirochaete demanded a surface lesion in these diseases, which must be deeper where the skin is drier. He supposed alopecia occurred because the roots of the hair were destroyed in that way. He did not think that bejel could be said to be an intermediate disease, because they saw negroes in Africa who had had yaws getting their typical syphilis afterwards.

With regard to Professor BLACKLOCK'S question as to the possibility of nervous involvement following insufficient treatment, he would point out that he had never seen yet any case which suggested nervous involvement in patients who had had insufficient treatment, and he had to admit that many thousands had had insufficient treatment. Tertiary lesions on the other hand, are provoked in this way.

He would ask whether Dr. HUDSON had thought of combined treatment, giving on the same day an intravenous injection of an arsenical and a large dose

of bismuth in the buttock, a treatment which is very successful in yaws; and whether he was able to give more than 30 cg. a week of bismuth metal without causing stomatitis.

Dr. William Corner (of Hove) said he was personally interested in Dr. HUDSON'S lecture because he had had experience of the disease when in the Iraq Health Service some years ago. Bejel was equally prevalent under the same name among the semi-nomadic Arab cultivators on the Tigris below Mosul and on the Lower Lesser Zab, the affected area lying about 200 miles East of Dr. HUDSON'S district and separated from it by desert inhabited by nomads proper. He would like to ask Dr. HUDSON what were his impressions with regard to the prevalence of bejel among these nomads who, if he remembered rightly, belong to the Shammar tribe.

Apart from hospital outpatients his experience was mainly gained by field work in villages in the endemic area. A feature of early cases was mucous patches in the mouth. In fact, mucous patches were so common that he was surprised that Dr. HUDSON has limited their appearance to the first year. Dr. HUDSON may have referred to these, however, as mucocutaneous relapses. He was convinced that practically 100 per cent. of cases had mucous patches in the mouth at one time or another. Another very common condition in adults was leucoplakia of the inner aspect of the cheek at the angle of the mouth. With regard to skin eruptions, late ulceration was very common but the early exuberant eruption of which Dr. HUDSON had shown some beautiful photographs he had found not common. In the average early case skin lesions seemed not severe and had to be looked for.

With regard to venereal syphilis of the towns he would like to ask Dr. HUDSON if, in his opinion, it is as virulent as European syphilis. His impression was that it was milder.

With regard to the name " franghi," he had always understood that in Arabic derivation it meant European, in other words, the European disease or disease of the Franks. Alternately, it might be the survival in Arabic, and thus in the East, of one of the old names for syphilis when it blazed out in Europe, namely, the " French disease." It would be remembered that the first mention of the word syphilis was in the description of the disease by Frascatorius in 1530, his writing being entitled " *Syphilis sive Morbus Gallicus*," about 50 years after its appearance. In either case, it indicates a western origin.

He would also like to ask Dr. HUDSON if he had come across references to a bejel-like disease in animals, particularly in horses.

The President (SIR ARTHUR BAGSHAWE): Before asking Dr. HUDSON to reply to the numerous questions, I would add my appreciation of the work

he has done. Dr. HUDSON has been working in a remote, isolated part of the world, and has been carrying on a big native practice as well. As a previous speaker has said, Dr. HUDSON has made a real contribution to knowledge.

Dr. Hudson (in reply) said that though 90 per cent. of adult Bedouins had bejel, only 15 or 20 per cent. of the total population had infectious lesions at any one time. If those cases were treated to the point of healing, and further ones treated as soon as they appeared, the number of new cases would drop very rapidly.

He had no knowledge bearing on the question of the production of late nervous sequelae by sub-curative antisyphilitic treatment. What evidence there was seemed to be inconclusive, and for his part he would risk the possibility of a few individuals getting neuro-syphilis in order to achieve the important objective of eradication of the disease from the community.

As to bismuth compared with intravenous arsenicals, he was convinced that sub-curative dosages of the latter were pharmacologically unsound and progressively ineffectual, while short courses of bismuth—though admittedly not ideal—produced the desired healing effect without risk to the patient and without prejudice to possible future treatment. For this reason, he had not chosen to combine the arsenicals with bismuth. A requisite of intravenous treatment was the systematic return of the patient, and this, in his experience with the primitive patient, had proved unattainable.

As to bismuth dosage, he had found that the normal adult could safely take 20 cg. of the metal as sodium bismuth tartrate, and 30 cg. of the metal as bismuth salicylate in the course of 1 week.

The administration by mouth of arsenic in pentavalent form might be effectual in the treatment of endemic syphilis, but a thorough trial of this treatment would require complete control of the whole population for a period of time.

Dr. HUDSON said that he had not seen adenoid hypertrophy of a unilateral character such as would justify reference to a chancre of the oral cavity. Adenopathy was a prominent feature of bejel, but bilaterally symmetrical.

In regard to certain points he wished to remove apparent misapprehensions. One of the photographs showed the earliest bejel lesion ever observed. It was a papule, and not a primary lesion in the sense of a chancre. This differentiation was to be made on immunological as well as on morphological grounds. There was, of course, a primary lesion in bejel in the sense of a *first* lesion, as there was in every eruption, and this first lesion resembled that of yaws in its extra-genital location, and in its apparent failure to exhibit " chancre immunity."

Another ambiguity arose in connection with the point as to whether the spirochaete of bejel could penetrate the choroid, infect the parenchyma of the nervous system and pass the placenta. Dr. Hudson insisted that it was quite

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possible that the spirochaete of bejel could under certain circumstances and in a certain proportion of cases do all of these things. His point was that in his experience with bejel he had not seen clear-cut clinical cases of these types. He thought there must be a threshold in the pathological process above which clinical signs and symptoms would appear, and he thought that under the epidemiological conditions of bejel the infection seldom rose to this level in the subject.

There was a sense in which it was true that yaws and bejel left the constitution unimpaired as compared with syphilis, and it was apparently in this sense that the expression was used in the differential table quoted. The statement was certainly debatable, however, unless there was agreement on the use of the terms.

The venereally acquired syphilis in the towns did indeed seem to be milder in form than the same in the West. There was close blood relationship between Bedouins and townspeople, and if there was any racial immunity the townspeople would share in it. It was also possible that certain adults who had acquired franghi had had bejel in childhood.

It was his impression that a large proportion of the *nomad* Bedouins of the Syrian Desert also had bejel, but exact figures were lacking as few of them sought treatment in the towns. Dr. CLAWSON, dental surgeon of the Iraq Petroleum Company, was making a study of the oral manifestations of bejel among the desert nomads.

He had several times been told by villagers that bejel came from animals, and he was shown a mare which was said to have bejel. The French veterinary surgeon, however, said that she had dourine. An ignorant people might be misled by the similarity of the lesions, their site and the mode of transmission.

It was true that it had not been easy to get together the photographs which had been shown ; they were the typical cases which had appeared at the clinic in the course of 18 months, which he had had the opportunity to photograph. The exuberant skin lesions were plentiful among the children, but unfortunately, few children were brought in for treatment.

Dr. HUDSON said he had stated his personal belief that bejel was a form of syphilis because he believed it was due to infection with *T. pallidum*; but he left the question open as to whether bejel was also in an intermediate position between syphilis and yaws, a view which would imply the fundamental identity of the three diseases. These two points of view were not necessarily mutually contradictory.

Dr. HUDSON, in closing, expressed his appreciation of the interest shown, and his thanks to those who had contributed to the discussion.

Since the meeting, the Secretaries have received the following letter from Colonel HARRISON who took part in the discussion :

DEAR SIRS,

In connection with the discussion on bejel, perhaps the following extract from the translation by Dr. HELEN WILSON of a paper by Dr. W. F. VELTHUYZEN, Medical Officer of Health, Amsterdam, which was read at the International Abolitionist Congress in Graz, Austria, in September, 1924, may be of interest and worth publication as an addendum to the discussion.

" No one doubts that from the epidemiological point of view there is a broad distinction between syphilis of sexual origin and endemic syphilis, which is in most cases extragenital, and propagated by means other than sexual relations, and to which consequently the name of venereal disease does not apply."

" The legislation of the Scandinavian countries shows the influence which this special form of extragenital syphilis may have on the construction and application of the legal enactments of a nation. Professor EHLERS, of Copenhagen, in his interesting pamphlet, '*Le Syphiloïde de Jutland*' (1923), develops the origin and history of the Danish legislation, dating from 150 years ago ; and it has a bearing on the struggle against the propagation of venereal diseases. We there read that formerly, owing to the insanitary habits of the Danish peasants, syphilis prevailed as an endemic disease in Denmark. The peasant family ate from a common dish, using a wooden spoon which was passed round. The spoon was cleaned after the meal and placed on a beam under the roof till the next meal, consequently primary infection of the mucous membrane of the mouth was frequent. The Bishop complained that a number of persons without noses presented themselves at confession, and Professor EHLERS points out that the destruction of the median cartilage of the nose, which leads to its disappearance is a frequent consequence of infection by the mouth. The doctors were impressed by the similarity of the symptoms to those of syphilis ; but the absence of the initial genital chancre, and the propagation of the disease by extragenital means, made them doubt its syphilitic character. This indecision gave rise to the word ' syphiloid ' by which they described it, signifying ' a disease resembling syphilis.' "

" Similarly in Sweden, at the beginning of the last century syphilis prevailed in endemic form among the peasant population and the statistics of POPAU, BELOUSSOW and others show that in 1884-5, in the governments of WLADIMIR, RYASAN and KWIRK respectively 91 per cent., 74 per cent. and 92·1 per cent. of the cases of syphilis were occasioned by means other than sexual relations. Again, Bosnia, Herzegovina and various other countries know syphilis as an endemic disease."

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peasants, living in ignorance of hygiene, is considered by the people not as a sexual malady, but as an infectious malady to which no shame attaches, like smallpox. This fact explains how it is that this form of syphilis loses its disgraceful character, and that it is possible to combat it in a way that is not possible with sexual syphilis, where there is a strong desire for secrecy. In the application of repressive measures compulsory notification, hospital treatment, compulsory or gratuitous treatment, no more resistance is met with than if it were some other infectious disease. This state of things explains the history of Scandinavian legislation on the subject of venereal disease. Moreover, the Scandinavian laws, drafted at the period when the three kinds of disease were confused, although more recently modified, are not entirely free from the initial error."

Yours faithfully,

L. W. HARRISON.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol XXXI. No. 1. June, 1937.

COMMUNICATIONS.

DESCRIPTION OF A NEW FILARIID FROM GROUND DOVES OF ST. CROIX, VIRGIN ISLANDS.

BY

DONALD L. AUGUSTINE, S.B., D.Sc.

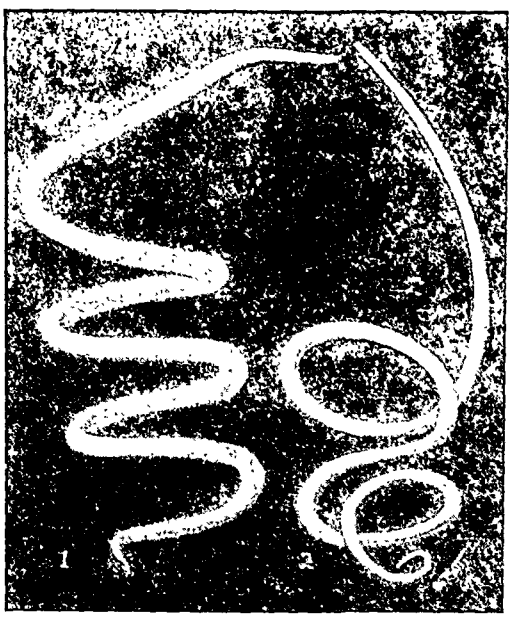
From the Department of Comparative Pathology, Medical School and School of Public Health, Harvard University, Boston, Massachusetts.

In his search for a suitable experimental animal naturally infected with filariids, Dr. F. W. O'CONNOR* discovered that blood of ground doves, *Columbigallina passerina nigrirostis*, of St. Croix, U.S. Virgin Islands, is frequently and usually heavily parasitized with sheathed microfilariae. Dr. O'CONNOR kindly sent the author a preserved specimen of one of these doves in which numerous adult filariids were found in a knotted mass directly beneath the peritoneum between the proventriculus and the gizzard. All appeared to be of one species. The smallest bit of tissue, particularly the lung, teased apart in a drop of water on a slide, contained hundreds of microfilariae. Later, during the summer of 1936, the author accompanied Dr. O'CONNOR to St. Croix, where further studies were made upon this parasite at the Municipal Hospital in Christiansted.

*Presbyterian Hospital, Columbia University.

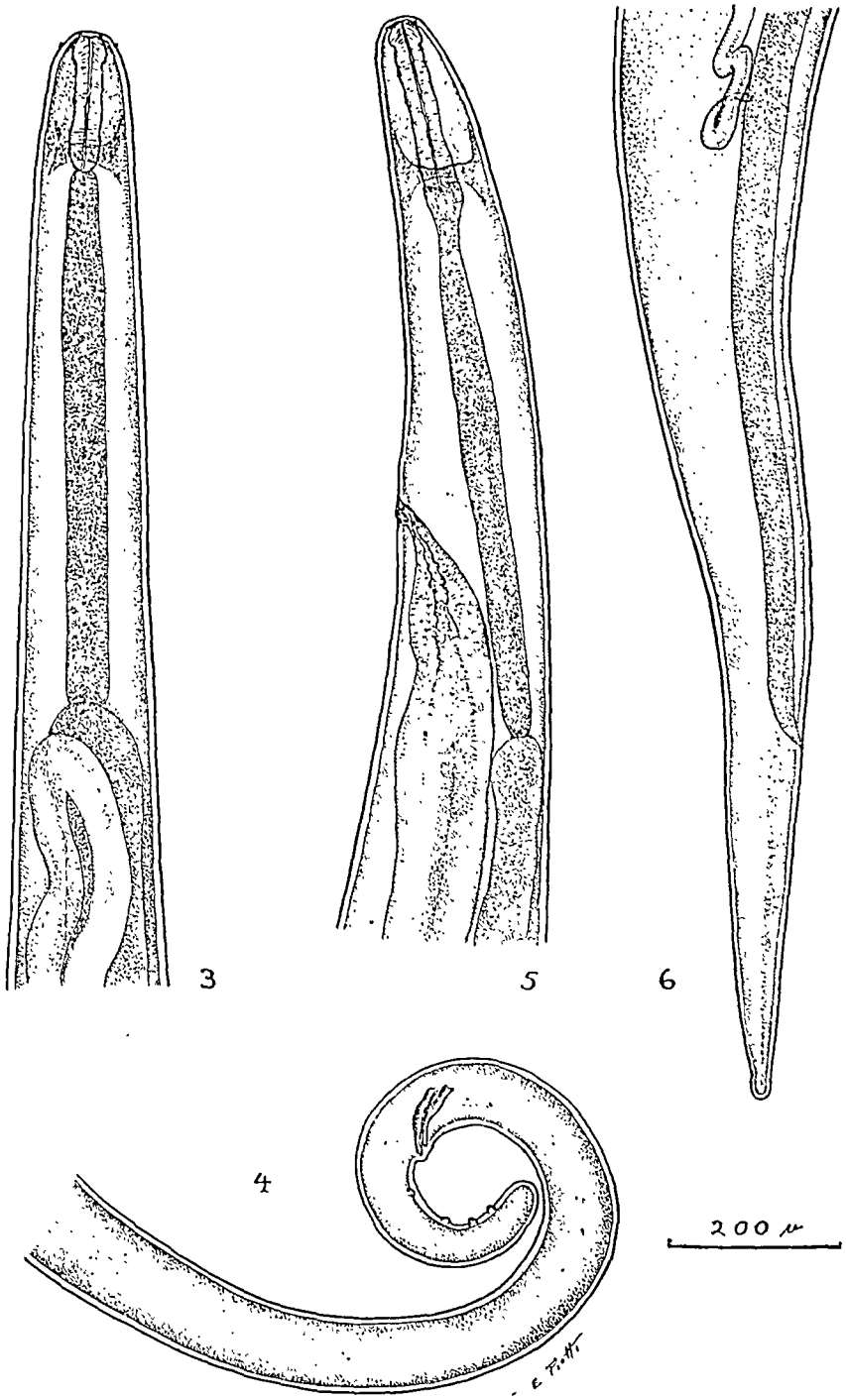
Description.—The following description is based upon the study of living specimens, and specimens killed in hot 70 per cent. alcohol and gradually transferred to glycerine. Microfilariae were studied alive in heparinized blood and in fixed dehaemoglobinized blood smears stained with methylene blue.

The adult parasite is a rather small, slender white nematode. The females are approximately twice the length of the males. The body is nearly uniform in thickness throughout its length but tapers anteriorly and posteriorly, terminating in rounded ends. The body assumes a loose spiral form when killed in hot 70 per cent. alcohol (Figs. 1 and 2). The cuticle is smooth. The



Vagrifilaria columbigallinae n.g., n.sp., camera lucida drawings of adult worms (× about 12).
FIG. 1.—Female. FIG. 2.—MALE.

mouth is simple and is surrounded by one pair of lateral and two pairs of submedian papillae. The papillae are small, inconspicuous and often scarcely perceptible. There is no buccal cavity. The oesophagus is cylindrical and approximately equal in diameter throughout its length. It is divided into two parts, a short, muscular anterior portion and a longer posterior portion which appears to be of a glandular nature. The intestine is somewhat bulbous at its juncture with the oesophagus. With the exception of the terminal portion of the



Vagrifilaria columbigallinae, n.g., n.sp. Morphology of adult worms.

[*For description see opposite page.*

vagina, the reproductive organs of both sexes lie posterior to the junction of the oesophagus and the intestine.

Thirty-nine out of forty-two ground doves were found infected with this parasite. From twenty-six to thirty-six adult worms were frequently encountered in a single bird. Besides occurring in the peritoneum, the adult parasites have been found in distended veins of the liver, spleen and pancreas, in the pulmonary vessels and the auricles and ventricles of the heart. They occur most frequently in the liver.

Male.—Males (Figs. 1, 3 and 4) are about 13 mm. in length with a maximum diameter of about 200μ. The oesophagus is from 750 to 800μ long and from 50 to 60μ in diameter. The anterior muscular portion is approximately 150μ long, with the nerve ring behind the middle of its length. The tail is somewhat digitiform, about 240μ long, curled ventrally, and bears four pairs of inconspicuous papillae situated approximately 80μ, 120μ, 150μ and 185μ respectively posterior to the cloaca. The two spicules are approximately similar in shape and size and measure from 70 to 80μ long, with a maximum breadth of about 15μ. There are neither pre-anal papillae nor is there a gubernaculum.

Female.—Mature worms, judged to be so by the presence of fully developed microfilariae in the uterus (Figs. 2, 5 and 6) measure from 20 to 23 mm. in length and have a maximum thickness of about 300μ. The oesophagus is approximately 1 mm. long and 50μ in diameter. The anterior muscular portion of the oesophagus is about 200μ long. The nerve ring is slightly anterior to its hinder end. The vulva is located at about the level of the middle of the posterior portion of the oesophagus. The vagina is trumpet-shaped and muscular. It passes directly backwards, soon giving rise to two straight, parallel uterine branches which largely fill the body cavity to about 1·5 mm. anterior to the anal pore, at about which level the oviducts arise. The ovaries are relatively short, somewhat coiled, and usually occupy the space between the posterior extremities of the branches of the uterus and the anal pore. Occasionally a loop of an ovary may extend beyond the anal opening. The uterus is crowded with microfilariae in varying degrees of development.

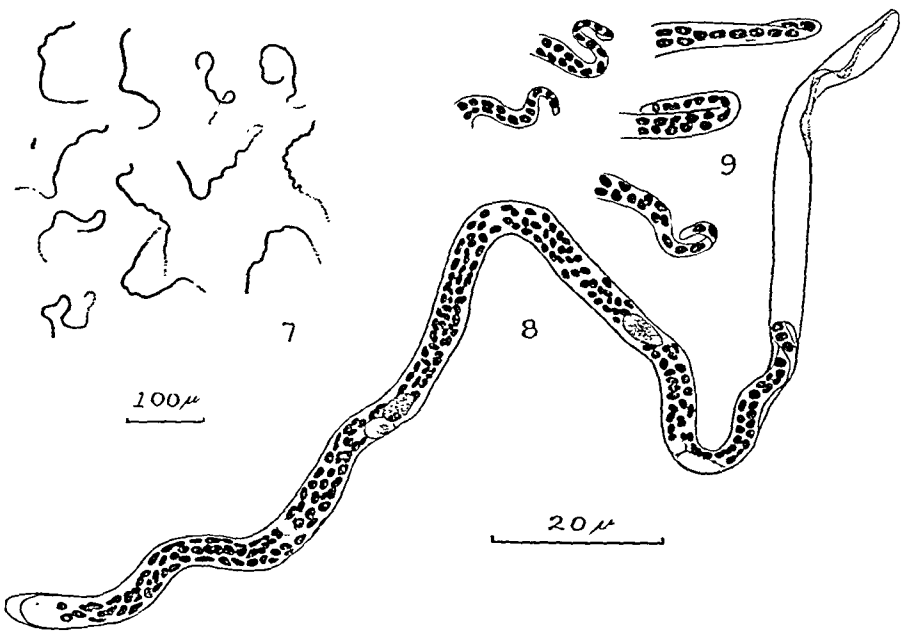
FIG. 3.—Anterior extremity of the male showing character of oesophagus and position of head papillae.

FIG. 4.—Posterior extremity of the male showing spicules and position of post-anal papillae.

FIG. 5.—Anterior extremity of the female showing position of vagina and vulva.

FIG. 6.—Posterior extremity of the female. The terminal portion of the posterior ovary is shown.

Microfilaria.—Microfilariae (Figs. 7, 8 and 9) in fixed and stained blood smears are enclosed in a sheath, which structure may extend from 40 to 70μ beyond the extremities of the body. The lengths of 30 measured specimens ranged from 146 to 200μ, exclusive of the sheath, with a diameter of about 7μ. The body characteristically assumes angular or spiral attitudes. The head end is rounded. The tail tapers to a somewhat rounded point. The tip of the tail is usually bent back upon itself, thus giving a blunt and sometimes a lobular appearance to the posterior end of the body (Fig. 9). The anterior tip is free from any structures for a distance of from 6 to 9μ. The nuclei are irregularly disposed in about three rows, except in the tail, where they occur in a single row, usually of 5 or 6 cells. The excretory cell is immediately posterior to the excretory pore. The following measurements of recognized fixed points from the anterior extremity are averages for 30 microfilariae. Nerve ring, 43μ; excretory pore, 65μ; excretory cell, 72μ; first genital cell, 116μ; anal pore, 139μ; total length 165μ. A clear area appears in the column of nuclei between the excretory cell and the first genital cell at a



Vagrifilaria columbigallinae n.g., n.sp., microfilariae.

FIG. 7.—Microfilariae. Showing characteristic attitudes of mf. in a stained blood smear.
FIG. 8.—Microfilaria. Showing disposition of nuclei and " fixed " points.
FIG. 9.—Posterior extremities of microfilariae.

distance of approximately 104μ from the anterior end. The cuticle appears to be without striation. The microfilariae are present in the circulation at all hours, but appear to be most numerous at night.

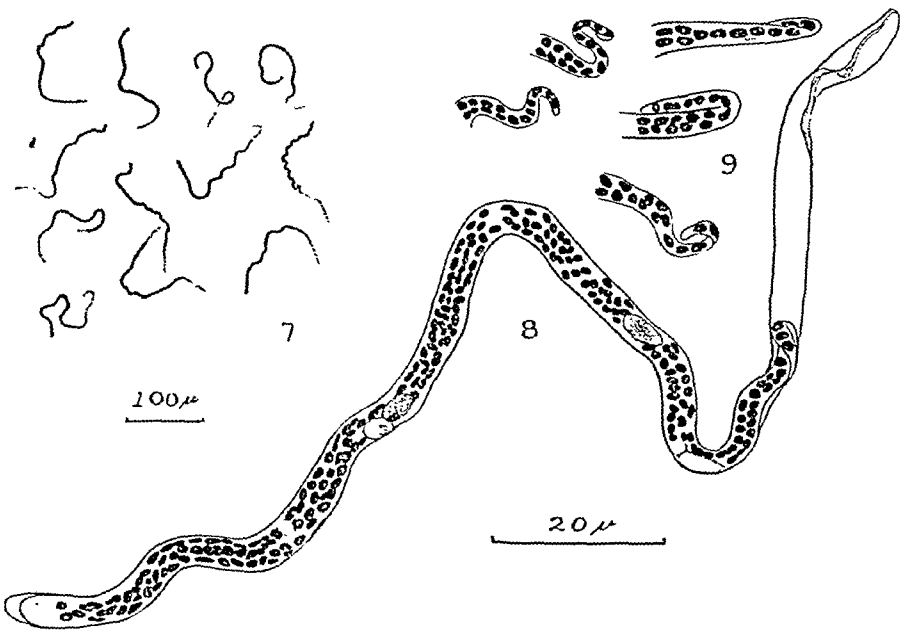
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It is obvious that our parasite belongs to the superfamily Filarioidea (Weinland, 1858) Stiles, 1907, and to the family Filariidae (Cobbold, 1864) Claus, 1885. In that the vulva is located in the oesophageal region, the cuticle smooth and the spicules approximately equal it can be referred to the subfamily Aproctinae, Yorke and Maplestone, 1925. We have been unable to refer our species to any genus which, at one time, has been placed with the Aproctinae.* It approaches the genus *Saurositus* Macfie, 1924, in that it has an unequally divided oesophagus. In *Saurositus*, however, the oesophagus is described as being clearly divided into two unequal portions, the posterior portion of which is much broader than the shorter anterior portion. With our species, the division of the oesophagus is not a conspicuous feature and it is equal in diameter throughout its entire length. Furthermore, in *Saurositus*, the vulva is located immediately posterior to the oesophagus, the tail of the female terminates in two lobes and lacks an anal opening; the tail of the male is blunt and short, with four pairs of pre-anal papillae and without post-anal papillae, and has a gubernaculum. Inasmuch as the genus *Lissonema* von Linstow, 1903, is now generally synonymized with *Aprocta* von Linstow, 1883, there are at least four species of *Aprocta* with unequal divisions of the oesophagus, namely, *A. narium* von Linstow, 1901, *A. rotundatum* von Linstow, 1903, *A. striata* Sandground, 1933, and *A. laevicutis* Sandground, 1933. In these species, the oesophagus is conspicuously divided into a short narrow anterior region and a longer, broad, posterior region, as in *Saurositus*. Our species further differs from them in the absence of lip-like elevations bordering the mouth, the absence of terminal portions of the testis and ovaries extending into the oesophageal region, and by their tapering, digitiform tail. The uterine branches of our species are filled with microfilariae in varying stages of development whereas the above-mentioned species of *Aprocta* are oviparous, the eggs being small and thick-shelled and containing an embryo at deposition. *Eulimdana clava* (Wedl) Founikoff, 1934, has been reported from the subcutaneous connective tissue of the neck, back and

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tail of carrier and domestic pigeons of Austria, France, Germany and Russia. The description of this parasite by FOUNIKOFF (1934) clearly shows that our specimen cannot be referred to this genus.

FILARIID WORMS REPORTED FROM GROUND DOVES AND PIGEONS.

A review of the literature shows that but little is known of filariids occurring in doves. KENNARD (1902) reported sheathed microfilariae from *Columbigallina passerina griseola* and *Leptotila rufaxilla*. Their descriptions are too meagre for identification. The adult parasites were not found. WALTON (1927) records a single, imperfectly preserved female filariid from *Columbigallina passerina passerina* in the Leidy collection of nematodes. The specimen was taken from a cyst in the hepatic region of the bird. It was impossible to identify the parasite, but it is considered by WALTON to be closely related to the genus *Pelecitus* Railliet and Henry, 1910.

Diagnosis.—It is believed that the present species is new and represents a new genus for which the name *Vagrifilaria* n. gen. is proposed. *Vagrifilaria* n. gen.—Aproctinae: mouth simple, without lips; two lateral and four submedian head papillae; cuticle smooth; oesophagus not conspicuously divided into two portions, both portions being equal in diameter; terminal portions of testis and ovaries posterior to the oesophagus; posterior extremities tapering and digitiform. Anus present.

Male.—Without caudal alae, without pre-anal papillae; four pairs inconspicuous post-anal papillae; spicules equal; gubernaculum absent.

Female.—Vulva in the oesophageal region; uterine branches parallel; microfilariae sheathed, in the blood. Parasites of the venous system and peritoneum of birds.

Type species.—V. columbigallinae n. gen. n. sp. ♂, 13 mm., ♀, 23 mm. in *Columbigallina passerina nigrirostis.*

Locality.—St. Croix, U.S. Virgin Islands.

The type specimen is to be deposited in the helminthological collection of the Museum of Comparative Zoology, Harvard University, and paratype specimens are deposited in the British Museum (Natural History) London.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

OBSERVATIONS ON LIVING "SHEATHED" MICROFILARIAE IN THE CAPILLARY CIRCULATION.

BY

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From the Department of Comparative Pathology, Medical School and School of Public Health, Harvard University, Boston, Massachusetts.

In a previous study (AUGUSTINE and DRINKER, 1935) it was shown that when blood heavily infected with microfilariae of *Dirofilaria immitis* is given intravenously to an uninfected dog the microfilariae appear within a few minutes in its lymph. Their escape from blood capillaries into lymph capillaries is accomplished, apparently, without appreciable injury to the capillaries. Later, an attempt was made to observe directly the manner of migration of microfilariae from the blood circulation into the lymphatic system (AUGUSTINE, FIELD and DRINKER, 1936). Numbers of *Microfilaria immitis*, isolated and concentrated, were injected intravenously into a bat previously anaesthetized with nembutal. The bat was then arranged for microscopical observation of the capillaries and lymphatics in the wings. Although no microfilariae were seen leaving the vessels or capillaries, it was discovered that they were exceedingly active within the capillaries; not being passively carried about with the blood stream, but actively migrating rapidly both with and against the blood stream, even in the larger vessels, provided these are small enough for alternate curves of the microfilariae to get a purchase on opposite sides of them. Their every movement had the effect of maintaining them in the circulation; 24 hours later, they were no longer seen in the circulation. They were then found concentrated in the liver and lungs, apparently having been filtered out of the circulation by these organs.

It was suggested that, perhaps, the observations applied only to unsheathed microfilariae, and that the sheaths of microfilariae of *Wuchereria bancrofti* and other similar forms might be a serious impediment to their progress in the circulation.

Opportunity to determine this very question was afforded the author during the summer of 1936 at the Municipal Hospital, Christiansted, St. Croix, U.S. Virgin Islands in the company of Dr. F. W. O'CONNOR.

EXPERIMENTS WITH *Microfilaria columbigallinae.*

The observations were made mostly on *Vagrifilaria columbigallinae* Augustine, 1937, a filariid discovered by Dr. F. W. O'CONNOR in the ground dove, *Columbigallina passerina nigrirostis* (AUGUSTINE, 1937). This microfilaria is sheathed; it is somewhat smaller than *Mf. bancrofti*, but in other respects the two are strikingly similar.

Doves were easily caught during dry weather in drop traps over water holes. It was not practical and seldom necessary to concentrate these microfilariae as done in earlier experiments (DRINKER *et al*, 1935). A single drop of blood usually teemed with microfilariae, and the amount of blood obtained from one dove was usually more than could be used in a single experiment. The doves were bled directly from the heart. To avoid clotting, the heart was swabbed with heparin, and a small amount of this was put in the syringe before bleeding.

Two species of bats of St. Croix were so heavily pigmented that clear vision of their capillaries could not be obtained. Frogs, *Rana pipiens*, brought with us from New York proved very satisfactory for the study. For an experiment a frog was anaesthetized with urethane injected into the dorsal lymph space. It was then mounted in position, the abdominal vein isolated and cannulated, the cannula pointing centrally. Undiluted, heparinized dove's blood, heavily charged with microfilariae, was introduced through the cannula, the vein was then tied, the cannula removed, the incision in the abdominal wall closed, and the feet of the frog spread and mounted on plasticene blocks in a large petri plate containing sufficient water to cover them. The capillary network was then viewed with a water immersion lens. A typical experiment was as follows :—

June 29th, 1936.

11.05 a.m.—Frog anaesthetized with urethane. Abdominal vein isolated and cannulated.

11.30 a.m.—Injected about 0·3 c.c. heparinized dove blood heavily infected with microfilariae into frog circulation through cannula. Cannula removed. Incision closed. Frog's feet mounted for microscopic observations.

11.50 a.m.—Numerous, exceedingly active, microfilariae observed travelling with and against blood stream in capillaries and in larger vessels. They cross capillary network from venous to arterial side and from arterial to venous side, back out of temporary obstructions (occlusions due to lodged white and red blood cells) seeking an avenue of escape with tail, turn about in the capillaries and proceed in opposite direction. Ten to fifteen microfilariae are frequently seen in " nests " (occluded capillaries). They are very active, knocking each other and the stagnated cells about in the effort to escape. Suddenly some distant barrier apparently was released and all microfilariae disappeared *en masse* into the active circulation. Contours of the vessels are not altered by movements of the microfilariae. The " sheath " is not seen on any microfilaria.

1.45 p.m.—No change—microfilariae active.

2 p.m.—0·3 c.c. saline injected to dorsal lymph space. Withdrawn after a few minutes and examined for microfilariae. None found.

2.10 p.m.—Frog (still mounted in petri plate) placed in cement court yard in full exposure to sun. Sky cloudless.

2.15 p.m.—Frog recovering from anaesthetic, given 0·2 c.c. 25 per cent. urethane in dorsal lymph space. Returned to laboratory for examination.

2.35.—Microfilariae active in capillaries. No microfilariae in lymph drawn from dorsal lymph space.
5.30 p.m.—Microfilariae in capillaries but noticeably fewer in number.
10 p.m.—No microfilariae seen in the capillaries.

June 30th.
8.45 a.m.—No microfilariae in lymph from dorsal lymph space. Anaesthetized with urethane, mounted for study of capillaries.
9.10 a.m.—No microfilariae seen in capillaries of either foot.
2.10 p.m.—No microfilariae in lymph from dorsal lymph space. An occasional microfilaria is seen in the capillaries *being carried with the blood stream*. Not active, movements limited to weak flexures of the body. Blood circulation strong.
2.45 p.m.—Frog placed in full exposure to sun. Frequently bathed with water to prevent drying.
3.30 p.m.—Returned frog to laboratory. No microfilariae from dorsal lymph space—none seen in the capillaries.
8 p.m.—No microfilariae in lymph or in capillaries.

July 1st.
9.25 a.m.—No microfilariae in lymph from dorsal lymph space. Frog anesthetized with urethane.
11.30 a.m.—Continuous search made for microfilariae, none seen in capillaries.
2.15 p.m.—No microfilariae in lymph from dorsal lymph space, none seen in the capillaries.
3 p.m.—Frog killed. Scrapings from lungs and liver negative for microfilariae. Tissues fixed for sectioning.

Experiments of this type clearly show that "sheathed" microfilariae actively and forcibly migrate through the capillaries, all their movements having the effect of keeping them in the circulating blood. Their travel in the capillaries is as effective as that of "unsheathed" microfilariae (AUGUSTINE and DRINKER, 1935; AUGUSTINE, FIELD and DRINKER, 1936).

Similar experiments were performed to determine if the activities of microfilariae obtained at night from the host differed from those taken during the day. Observations made from 10 p.m. to midnight revealed no apparent difference.

Living, gravid females dissected from livers of ground doves were placed directly in the dorsal lymph space of frogs. Living worms, embedded in the connective tissue of the dorsal lymph space were recovered 2 days later. Fluid from the dorsal lymph space contained living and degenerated microfilariae and, in one experiment, a single active microfilaria was found in blood taken from the ventral vein. No microfilariae were ever observed in the capillaries of these frogs; probably there were too few in the circulation.

EXPERIMENTS WITH *Microfilaria bancrofti*.

Attempts to perform similar experiments with *Mf. bancrofti* were unsatisfactory. Blood infections encountered in patients at hospitals of Christiansted, Frederiksted, and at a home for the aged, were so light that concentration of the microfilariae was necessary. They proved exceedingly delicate, and did

not survive haemolization and other steps employed in the process of concentrating *Mf. immitis* (DRINKER *et al*, 1935). Seven hydroceles aspirated at the hospital in Christiansted were negative of microfilariae. It was, thus, at no time possible to obtain living *W. bancrofti* in quantity for our experiments.

In one experiment about 4 c.c. of heparinized blood containing *Mf. bancrofti* were injected slowly into the dorsal lymph space of the unanaesthetized frog at 11 o'clock at night. At 9 o'clock the following morning the frog was anaesthetized with urethane and the hind feet mounted for microscopical study. The circulation was strong, but no microfilariae were noted in the capillaries. Observations were continued intermittently until 5 o'clock in the afternoon. No microfilariae were seen. The frog was then killed and the organs removed separately. Several living and apparently normal microfilariae were found in scrapings from the liver. Although the experiment failed in its original purpose, the finding of microfilariae in the liver is significant. It indicates that these microfilariae are, as were those of other species studied, filtered out of the circulation into that organ. Histological study of the sections of the tissues removed and fixed at autopsy indicates that microfilariae are also finally disposed of in the liver.

In our previous experiments with *Mf. immitis* it was found that they readily escape from the blood capillaries and appear in the lymphatics. This phenomenon was not observed in any of the present experiments with *Mf. columbigallinae* or *Mf. bancrofti* in frogs. Whether these microfilariae do penetrate tissue remains unknown. In one experiment a *Mf. bancrofti* was recovered in blood from the abdominal vein of a frog 18 hours after injection into the dorsal lymph space. Its presence in the blood circulation may have been largely due to passive rather than active migration.

HISTOLOGY.

Frogs killed from 18 to 31 hours after injection with *Mf. columbigallinae* or *Mf. bancrofti* show these distributed throughout the liver but none in the other organs. They lie in the liver sinusoids and occasionally in the veins. Many appear to have been alive and active at the time of fixation—as judged by the normal position of the body nuclei, smooth cuticula, and characteristic curves of the body indicative of active progression. Others were apparently dead and showed degenerative changes—the nuclei distorted the cuticle crumpled, and the body lacking the characteristic contours of normal microfilariae. There was no cellular infiltration.

Livers of frogs killed 5 days after injection with microfilariae showed no organisms. There were however, scattered throughout the liver, fairly numerous small areas of lymphocytic infiltration which may possibly represent a late reaction persisting after the disappearance of microfilariae. If such be the case, the earlier cellular response was not obtained in our limited series.

The question arises whether microfilariae are actually killed in the liver or if they die elsewhere in the body and are then carried by the circulation to the liver where they may be finally disposed of. The finding of feeble forms being carried passively in the circulation 27 hours after injection into the system rather suggests that this may occur. In all these and earlier experiments there was an early disappearance of the microfilariae from the circulation; 30 hours after injection into the circulation they were found concentrated in the liver where clearly degenerating forms were encountered and none in other organs. This may be the result of being in an abnormal host. On the other hand, it may indicate that many microfilariae are destroyed daily in the normal host.

"Sheathed" Microfilariae.

One object in the present study was to determine what hindrance the sheath might cause microfilariae in their migration in the circulation. Constant search for this structure on microfilariae in the capillaries failed to detect it. Neither was it visible on *Mf. columbigallinae* in fresh heparinized blood nor on microfilariae teased directly from tissue. Occasionally a very slight clear area was detected at the posterior extremity of *W. bancrofti* as it thrashed about in unclotted blood smears, which area may have been a space between the tail of the larva and a "sheath." The formation of the "sheath" could always be followed on the slide as the medium surrounding the larva dried. The process was repeatedly observed on *Mf. bancrofti* and *Mf. columbigallinae*. The larva is mechanically held in the ever thickening medium. In its efforts to push on and back out, the once close-fitting, inconspicuous outer covering is continually stretched until complete drying of the preparation prevents further movement of the microfilaria, which then perishes. It is quite possible that some stretching of the sheath may occur in the circulation at times when microfilariae are temporarily held in the capillaries. In such circumstances, however, the stress upon the outer membrane would never be as great or as prolonged as in a drying blood film. From the present observations, it appears doubtful if there are ever many "sheathed" microfilariae in the circulation.

These observations raise a question as to the exact nature of the "sheath" of microfilariae. The current conception that the "sheath" is the modified vitelline membrane seems to be based largely on the observation of Penel (1904) on *Loa loa* obtained at autopsy on a negro from the west coast of Africa. The adult worms were dead at the time of their removal from the cadaver. The development of the microfilariae was observed on specimens removed from different levels of the uterus and mounted in 20 per cent. formalin. The vitelline membrane was noted on eggs containing embryos and on some of the microfilariae the sheath extended for a distance of from 30 to 40μ beyond either extremity; in others, however, the larva so completely filled this envelope that it could be detected only at points of curvature of the body.

Developing microfilariae from the uterus of *Vagrifilaria columbigallinae*

clearly show the vitelline membrane surrounding eggs containing coiled larvae, but none of the microfilariae from the vaginal region show any evidence of a sheath. From this same region of the uterus are found accumulations of crumpled, hyaline objects, similar in size and shape to the membranes enclosing coiled embryos taken from a higher level of the uterus. These we interpret to be discarded vitelline membranes of mature microfilariae.

It appears, therefore, that the "sheath" on the species of microfilariae studied is comparable to that of infective hookworm larvae—namely, the result of an incomplete ecdysis. Although all nematodes undergo ecdysis, only a relatively few have developmental stages known as "sheathed" larvae. The absence of the sheath on microfilariae of several species cannot be explained at this time. It may be that the development of such forms is slower than that of "sheathed" microfilariae and that the formation and shedding of this cuticle takes place only in the body of the insect host. A careful study of the embryology and later development of "sheathed" and "unsheathed" microfilariae would be of decided interest and value. We can conclude, however, from the present study that the "sheaths" of *Mf. columbigallinae* and *Mf. bancrofti* as known in fixed and stained blood smears become apparent by technical procedure, but are indiscernible on microfilariae while in the circulation.

SUMMARY.

1. Experiments are reported in which the so-called "sheathed" microfilariae of *Vagrifilaria columbigallinae* have been studied in the capillary circulation of frogs. It has been shown that "sheathed" microfilariae actively migrate through the capillaries and that their behaviour in the circulation is identical with that of the "unsheathed" microfilariae of *Dirofilaria immitis*.

2. It has been shown that microfilariae are filtered out of the circulation by the liver after 17 to 30 hours, and histological evidence indicates that microfilariae are finally disposed of there.

3. *Mf. columbigallinae* injected into the circulation of frogs was not observed to enter the lymphatics.

4. The sheath, the apparent envelope surrounding microfilariae of several species in fixed and stained blood smears, is indiscernible as a distinct structure on these microfilariae while they are in the circulation.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

BANCROFTIAN FILARIASIS AND THE RETICULO-ENDOTHELIAL SYSTEM.

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I.—INTRODUCTION.

The position taken here is that two different mechanisms cause the rise and the fall of the microfilarial tide of Bancroftian filariasis; the mother worm's parturition puts microfilariae into the blood through the lymph current, the host's reticulo-endothelial system takes them out of this and destroys them. In other infections with endoparasites a dualism is not questioned—the female intestinal worm puts her eggs into the host's intestine, the intestinal muscles get rid of them; the female trichinella puts her young into the intestinal wall, the reticulo-endothelium mainly of striped muscle shuts them off from doing the host further harm. Yet this principle has, in effect, been denied for

Bancroftian filariasis; when I reasoned (1929) that the rise of its microfilarial tide was caused by the mother worm's parturition—so challenging MANSON's view (1882) accepted for some 50 years, and upholding that of MYERS (1881) almost forgotten for as long—and even when O'CONNOR had produced evidence, covering many hours of the twenty-four, that female worms in the same host give birth to their young simultaneously, it was said that what mattered was a demonstration of the mechanism which causes the fall of the tide. Active critics have not repeated O'CONNOR's extensive investigations, but have unhelpfully stressed our ignorance of the even less studied mechanism which takes microfilariae out of the blood.

Getting beyond such vague terms as " foreign body giant cells," this paper is a first attempt to suggest that the reticulo-endothelial system, taken as an entity, is the structure which causes the fall of the microfilarial tide.

II.—THE RETICULO-ENDOTHELIAL SYSTEM.

1. SOME PERTINENT POINTS.

In their summary of knowledge of this system, HADFIELD and GARROD (1934) tell that its mobile cells have two origins—the lymphocytes of lymphoid tissue, and the reticulo-endothelial cells of liver, spleen, lymph nodes, bone marrow and connective tissue—that among their gradations of form are plasma cells, macrophages, giant cells and fibroblasts; and that by them the body gets rid of protozoa and of bacteria of the tubercle type. Of helminths they say nothing; yet these very cells are prominent round helminths within bodily tissues and are strikingly so in Bancroftian filariasis.

For example, AFRICA and GARCIA report on ascaris eggs which had been long enough in the peritoneal cavity of a woman (1936a) and of the monkey *Macacus cynomolgus* (1936b), to become embryonated. In the peritoneum were tubercles having in their centres these eggs being devoured by giant cells, the other constituents in the tubercles being round cells, endothelial cells, fibroblasts and fibrous tissue, but no eosinophils. I have many sections made by O'CONNOR's staff of the livers of the ground dove of the Virgin Islands, *Columbigallina passerina nigrirostis*, showing giant cells, with or without enclosed microfilariae, Fig. 1, from worms* living in the blood vessels of that organ. In infection with *Wuchereria bancrofti*, CRUICKSHANK and WRIGHT (1914) noted in lymph vessels and nodes a proliferation of endothelial cells with fibroblasts while near adult worms even in lymph nodes were giant cells: observations, confirmed and amplified by O'CONNOR, the full importance of which seems unrecognized.

The fibroblast, and blockage of the system, need special consideration. Of *fibroblasts* HADFIELD and GARROD say that only these end cells seem capable of indefinite perpetuation in tissue culture, which surely suggests that persistence of a stimulus, which has brought them into being in the host's body, will easily cause much fibrosis; the conditions suited for their growth are considered

**Vagrifilaria columbigallinae*, Augustine, 1937.

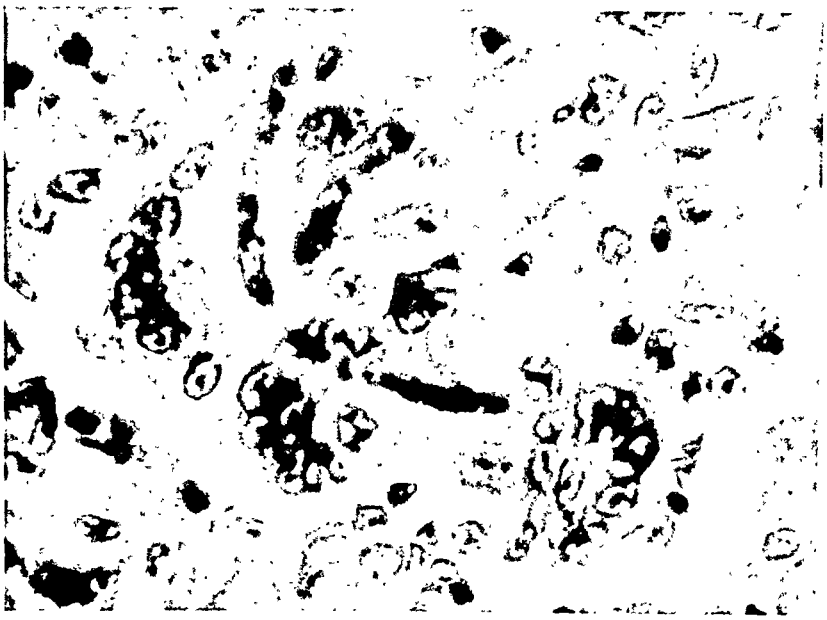


FIG. 1.—Photomicrograph of dead and degenerating *Mf. columbigallinae* in giant cells of liver of ground dove.

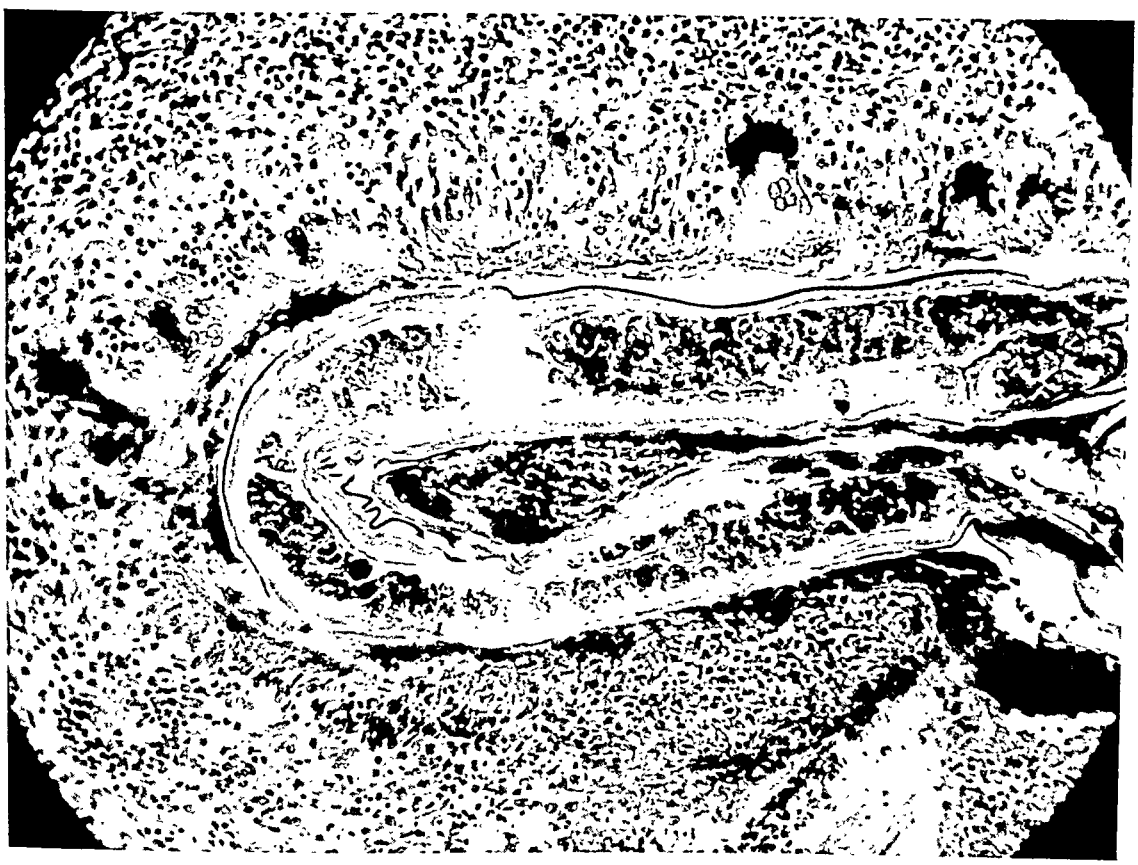


FIG. 2.—Giant and other reticulo-endothelial cells ranged along a degenerating female *W. bancrofti*.

Photographs given by Prof. F. W. O'Connor.

To face page 62.

later. Of *reticulo-endothelial blockage* these writers note that repeated injections of dyes and particulate matter so over-load the system's active cells that they seem incapable of movement or any other activity; and that by such injection combined with splenectomy (which removes much reticulo-endothelium) the overworked system may be blocked and unable to defend the body. They give this example: the spirochaete of relapsing fever cannot live in normal mice, but kills 90 per cent. of blocked animals. There are three reservations as to blockage of the system: solid particles and chemicals in solution have different effects; while a large dose of blocking material may inactivate, a small dose may stimulate; complete blocking has proved impossible. Blockage is a serious matter for a host animal and may be the chance of its lifetime for a parasite.

2. THE LYMPH DAM.

An infective larva of *W. bancrofti* entering man's skin has two conveyances at its disposal, the blood escalator and the lymph escalator (LANE, Discussion, O'CONNOR, 1932). Should its boring take it to the second it is carried away by the lymph current, but may be held up somewhere along the length of a lymph vessel or where this fans out before entering the nearest lymph node; and in one of these spots it may become adult. But adult worms are common in the scrotum, which an infected mosquito must rarely bite; so here the reasonable explanation is that the infective larva, entering somewhere else, has got on to the blood escalator as the hookworm larva often does, and been carried round by it till it has come to the capillaries of some part which " suits " it (in this case the testicle). Here it leaves blood capillaries (perhaps they are taking up from the tissue something with a taxis for it), gets into tissue fluid, so into a lymph vessel, and there becomes adult.

Round the *adult* worm, while to all appearance living and healthy, the endothelium may become swollen by new reticulo-endothelial cells (giant cells may be conspicuous, as in Fig. 2), and the worm may die from stoppage of the lymph flow on which its life depends, or perhaps from toxic excretions by the cells. The dead worm often becomes calcified, and in the end fibroblasts make round it a concentric web of fibres, a " parasitic onion."

Microfilariae, too, bring into being new cells of this system. With an adult in the testis obstructing the lymph flow, this, it seems, runs towards the tunica vaginalis, near whose angle of reflection a projecting granulation forms made up of lymphocytes, macrophages and giant cells with an occasional polymorph, and among the cells are microfilariae—normal, partly calcified but for the rest of their length with seemingly normal nuclei, with nuclei becoming poorly stained and disarranged—and empty cuticles having cell remnants within; a few of the disintegrating microfilariae are partly inside giant cells (as in Fig. 1), the rest are extra cellular. Round microfilariae in cortical sinuses of a lymph node are many macrophages and fibroblasts, and the microfilariae may be

undergoing extra cellular digestion with ghostly nuclei, or calcification in whole or part; by the presence of these unusual bodies and by contraction of the fibrous tissue which they form, the lymph is dammed and there results a hypertrophy and dilation of the afferent vessels and the usual ready formation of new lymph channels in this new fibrous tissue; so the node becomes a varicose lymph node in miniature, with islets of lymphoid tissue set in wide fibrous surrounds riddled with lymph vessels which bypass the active elements and take microfilariae safely to the next node*; this will go on node by node till microfilariae from a mother worm make their way, zigzag and slow but little molested, into the blood stream.

(*i*) *The Latent Period.*

Between the date on which microfilariae are first born in a part and that on which they first get into the circulating blood there will then be a latent period which should be shortest when a mother worm has few nodes between it and the great veins, or when many mother worms have matured about the same time in the same lymphatic drainage area—when defences are shallow or bombardment heavy; but for that, I think, evidence has not been sought.

The earliest ages at which microfilariae have been reported in the blood, by Commissions from the London School of Hygiene and Tropical Medicine, have been: 14 months in British Guiana (ANDERSON, KHALIL, LEE and LEIPER, 1924); 5 years in Fiji (BAHR, 1912), though a girl of 2½ had enlarged epitrochlear glands but no microfilariae; 4, 4 and 5 years in three groups of the Pacific Islands (O'CONNOR, 1923), while in them the percentages of those under 16, and 16 and over, with microfilariae in the blood, were respectively 9 and 46, 8 and 36·7, and 11 and 42. These are not necessarily the earliest ages at which microfilariae reached the blood; prenatal infection may perhaps take place; susceptibility to infection may unexpectedly increase with age; and there is an interval of apparently unknown length between entry of an infective larva and its maturity with birth of microfilariae. But, with all these doubts, the anatomical findings leave little question that till the lymph nodes between a mother worm and the great veins have been bypassed, microfilariae will not reach the blood—that there is certainly a latent period so caused.

To sum up this stage, there is proof of destruction of microfilariae, it may be for months or years, before any get into the blood; and even after some have done so, this will go on as long as lymph on its way to the blood takes others through active lymphoid tissue. It follows that the numbers of microfilariae counted in the blood are no measure of those which are being born, still less of the mother worms which bear them—another vexation for tidy minds which treat the clinical medicine of helminthiasis as a branch of lower mathematics.

(*ii*) *Elephantiasis.*

A most striking and distressing effect of the lymph dam is elephantiasis. Nearly 25 years ago LOW (1913) noted that it may come on without previous

*In O'CONNOR'S experience microfilariae are often seen in the afferent channels of a normal or nearly normal lymph node, but he has not seen them in the efferent channels.

lymphangitis* ; the experience of O'CONNOR and HULSE (1935) of 301 cases is the same. These conclusions are based on medical histories given by persons perhaps of poor intelligence, so that some may doubt the wisdom of taking them at face value. The experimental work in Professor DRINKER's laboratory at Boston, U.S.A., puts doubt at rest.

MILLER, SAYERS and YANT (1935) injected " dust " into the peritoneal cavity and found that it fell into three groups—the first inert and unchanged in inert tissues, the second soluble and disappearing, the third (including quartz and highly silicaceous chert) persisting and causing cellular proliferation and fibrosis. In the previous year it had been shown by DRINKER, FIELD and HOMANS (1934) that elephantiasis was brought about experimentally in dogs by repeated injections into their lymphatics of quinine solution (with its reputation for closing varicose veins) and of a watery suspension of fibrosing particles of crystalline silica having a mean diameter of 1μ. Repeated injections are needed, for with blockage of one set of lymph vessels new ones form and have to be closed ; so quickly do they form that after cutting through all tissues of a thigh except the femur, the femoral artery and vein, and the sciatic nerve, and removing the inguinal and popliteal lymph nodes, the lymph current becomes normal in about a week.

After these repeated injections into different lymph vessels of a limb, elephantiasis appears ; it may do so without lymphangitis but an attack of this quickens its oncome. Is this lymphangitis helminthic or bacterial ? To the answer the Boston workers point the way.

In the oedema fluid of elephantoid dogs they found haemolytic streptococci if examination was made not later than 12 hours after a lymphangitic attack began, but none were present between attacks unless septicaemia had set in (DRINKER, FIELD, WARD and LYONS, 1935) ; reports of failure to find them later in an attack have mislead myself and others into the conclusion that its cause was helminthic. Again, when DRINKER and his associates injected (in unit amount of 0·3 c.cm. per kg.) a culture fluid of this streptococcus, they caused fever and leucocytosis in normal dogs when each unit had in it 33,000,000 streptococci, in an elephantoid dog when it had 8 only.

Elephantiasis may make a dog 4,000,000 times more susceptible to haemolytic streptococci than is a normal dog. Moreover, on that composition of lymph which best makes for elephantiasis the Boston work has shed much light.

The percentage of protein in the hind leg lymph of fifteen normal dogs was between 0·7 and 5·71 with mean of 1·84 (DRINKER and FIELD, 1933, p. 72). Its mean in the blood serum of eighteen dogs was 7·33. These writers hold that from all blood capillaries there pass into the tissue-spaces water, salts and sugar in the concentrations in which they are present in the blood, and that these readily pass back into the blood stream ; proteins pass out of blood capillaries with difficulty and do not get back. Accordingly " if lymphatic obstruction occurs in the presence of a normal blood circulation, an abnormally high concentration of tissue fluid protein must result. . . . It would be of interest to obtain true lymph from cases of elephantiasis." Later they (DRINKER, FIELD, HEIM and LEIGH, 1934) did so from fifteen dogs deliberately made elephantoid, and its percentage of protein was from 2·25 to 4·58 (mean 3·61), and disintegrating cells were always plentiful ; with lymph obstruction the percentage of protein in tissue fluid was doubled.

*After reading this O'CONNOR comments, " In 1784 in his ' Glandular Diseases of the Barbados,' HENDY emphasizes this fact, describing cases and giving confirmative results of a questionnaire which he sent to his colleagues in the island."

The significance of this (DRINKER, FIELD and HOMANS, 1934) is that tissue fluid with a high protein content and with disintegrating cells is a culture medium giving excellent growth of connective tissue cells *in vitro*, in other words, a plentiful end-supply of fibroblasts.* But in Bancroftian filariasis the conditions will presumably make for an even better culture medium for fibroblasts since microfilariae are undergoing dissolution in lymph nodes with, surely, the setting free of more protein and so the quicker growth of fibroblasts; and when in addition there is acute inflammation this is more so.

As DRINKER and FIELD (1933, p. 106) put it, " As a region becomes inflamed the blood capillaries dilate and undergo an increase in permeability which may readily be so great as to permit the outflow of blood plasma. . . . From a region of inflammation it is easy to collect a large volume of lymph which is extremely rich in protein." This explains how it is that though elephantiasis may come on without inflammatory attacks, its progress is much quickened by them. So there is set up a vicious circle, increased obstruction making conditions better for increased fibrosis, and this in turn causing more obstruction.

But there is another side, true for dogs and presumably for man—the effect of leaks of lymph from an elephantoid limb. " In some animals they have been so persistent and extensive as to keep the protein concentration of the oedema fluid very low, and in such cases the permanent fibrotic enlargement of the part is not impressive." (DRINKER, FIELD and HOMANS, 1934.) The deliberate production of leakage in man to prevent increase in elephantiasis means steady loss of protein and the opening up to streptococci of a tissue with lowered resistance to them. This condition was clearly present when SAMPSON HANDLEY (1909) made lymph from an elephantoid leg drain into the lymphatics of the iliacus along the track of silk threads leading up from the foot; the circumference of the ankle lessened by a third, but acute inflammation soon set in.

There is then anatomical evidence that the reticulo-endothelial system effects great destruction of microfilariae before they get into the blood and that by this very act it becomes the cause of elephantiasis.

III.—THE MICROFILARIAL TIDE IN INFECTION WITH *Wuchereria bancrofti*.

As noted, the rise and fall of the microfilarial tide, it is held, are caused by different mechanisms, the rise by simultaneous timing of the cycle of parturition in the mother worms, the fall by the arresting and destroying action of the reticulo-endothelium of the host.

1. THE CAUSE OF THE RISE OF THE MICROFILARIAL TIDE.

Though beyond the title of this paper, yet for clearness some account of this must be given. Briefly (LANE, 1929) I came independently to the same

*It seems reasonable to suggest that, in conditions so favourable for their development to fibroblasts, the mobile cells will mainly develop into these end cells, and that in this way there may come about a lessening of general phagocytic action, and so a greater opportunity for bacteria to gain a footing in the body.

conclusion as had MYERS (1881) that, contrary to the view of MANSON, *Microfilaria bancrofti*, which is anchorless, was unable to harbour by day in the great currents of the heart and big vessels; nor did it do so in capillaries by some posture absent on a slide, or by some selective contraction of capillaries which at the same hour kept back *Mf. bancrofti* and let through *Mf. loa* and *Mf. perstans*; moreover such a haunt was undiscovered after 50 years' search; again, for microfilariae to keep out of the blood when the vector is not biting was of no advantage to the species. So the conclusion was come to that the rise of the microfilarial tide in periodic Bancroftian filariasis was due to simultaneous parturition by female worms. This hypothesis was tested by O'CONNOR, whose findings have been clearly put by HINMAN (1935b) in saying that there had been given " a demonstration of periodic variation in the contents of the uterus of female *W. bancrofti* by O'CONNOR and his associates (1929, etc.). The same stage of development was invariably found in all female worms from the same host. This is timed so that the microfilariae are all born about midday and shortly thereafter the uterus is found empty and the worm collapsed and wrinkled."* O'CONNOR's evidence is based on material from the living or dead body with the times of operation or death spread, not yet quite evenly, over the twenty-four hours; and it is that in the periodic infection a rapid parturition takes place about the same time in all female worms found in the same body or excised tissue; and that there is a lag of 10 or 12 hours between the birth of microfilariae and their high tide in the circulating blood, its natural explanation being the slow carriage of microfilariae by the lymph current. When they are present in the blood at low tide, it may be that somewhere in the body this current is unusually slow, that some female worm has mis-timed her parturition, or, as will be seen, that the removing mechanism is overworked, the reticulo-endothelial system blocked.

(*i*) *The Significance of the Spent Worm.*

The stages of intra-uterine development seen by O'CONNOR are these.

> The emptied uterus and branches steadily fill from the ovaries until the condition is one of packed, parallel embryos anteriorly, embryos coiled in the egg membrane behind this and further back eggs less and less segmented. With parturition there are expelled with some suddenness straightened and still coiled embryos and even some eggs, all these may be seen in the nearest lymph node, and the mother worm becomes empty and spent. Then this 24 hour cycle begins again.

It is because in periodic Bancroftian filariasis O'CONNOR has found that these changes take place in all female worms in the same human body about the same hour that the whole series has been traced and the significance of the spent worm made clear.

*The wrinkling of the worm is perhaps caused by preservation and hardening for sectioning.

(*A*) *The Spent Worm in Infection with* W. bancrofti *in Fiji.*

Intra-uterine development in the non-periodic form of *W. bancrofti* seems to go on in Fiji step by step as in the periodic form, but the worms in the same host do not, if one may say so, keep step. BAHR had not that help in recognizing the spent worm which O'CONNOR's work has since given, and his puzzling Worm F. (BAHR, 1912) I judge to have been in this state. It was 50 mm. long and had in its uteri eggs but no more advanced states of intra-uterine development, and was classed as "adult" in Table I, as "apparently immature" on p. 9, and as "immature" in Appendix V, p. 87.

(*B*) *The Spent Worm in Infection with* Dirofilaria immitis *in the Dog.*

D. immitis lives normally in the blood stream, and the young are born into the blood; if so they will be seen in a large enough sample of it immediately afterwards. This is HINMAN's description (1935b) of the state of female worms. "With the exception of only an occasional female which might be accounted for by immaturity or sterility, at any hour of the day including midnight, the females always contain tremendous numbers of active elongated microfilariae, as well as coiled embryos within the shell and also segmented and immature ova. . . . Thus it is concluded that in *Dirofilaria immitis*, contrary to *Wuchereria bancrofti*, no evidence of cyclical parturition has been found." As the experiments were put through such evidence was surely impossible to get—for this reason.

The curves (and heights) of the microfilarial tides in the two infections are very different (Fig. 3); in an ordinary blood sample there are at low tide in the first few or no microfilariae, in the second perhaps half as many as at high tide; thus in the report of WARRINGTON YORKE and BLACKLOCK (1917), the numbers per cubic centimetre of *Mf. bancrofti* at high and low tides were 12,850 and 50 (257 to 1), those of *Mf. immitis* given by HINMAN (1935a) were 47,000 and 16,235 (3 to 1), and by KHAW and CHEU (1936) 16,000 and 2,600 (6 to 1). If then the rise and the persistence of the microfilarial tide in infection with *D. immitis* are caused by parturition of worms living in the heart*, that takes place at all hours but more so at that of high tide; and, anatomically, spent worms will be found all round the clock, but mostly so at the hour of high tide in that particular dog.

The essence of the matter is this. The presence of spent worms at certain hours is an important part of the evidence, accepted by HINMAN, that the microfilarial periodicity of infection by *W. bancrofti* is due to parturition by mother worms; yet he does not use it in considering the imperfect periodicity of infection with *D. immitis*, but puts down the emptiness of these worms to their youth or old age; that conclusion surely may not be come to until the

*Occasionally they have been reported from sites outside the blood vascular system. If identification was right there would in such cases be a lag between parturition and the appearance of microfilariae in the circulating blood. The need for care in drawing confident conclusions as to the cause of periodicity in this infection is great. The problem is not the simple one it has been thought to be.

likelihood that they are spent worms has been set aside ; that it seems, has not been done. The work of KHAW and CHEU (1936) on treatment of infection

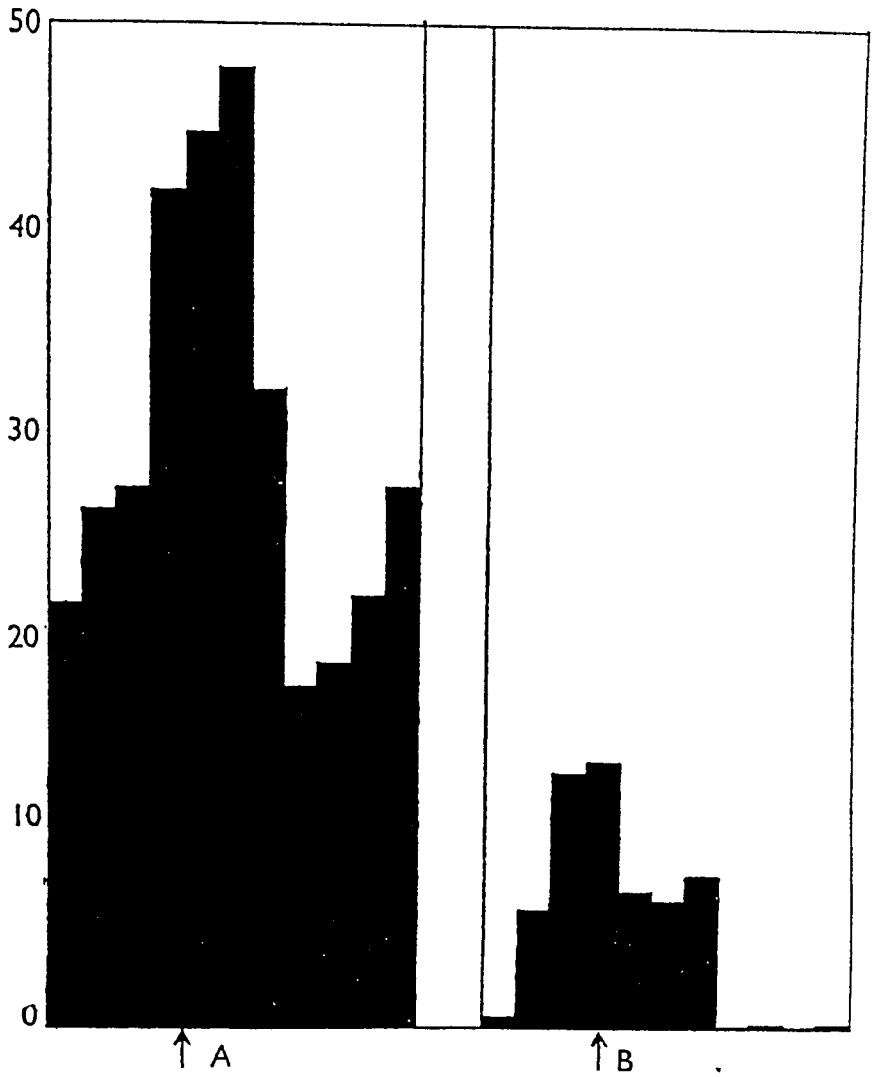


FIG. 3.—Microfilarial tides measured in thousands of microfilariae to the cubic centimetre. The arrow indicates midnight.

A. *Mf. immitis* in dog, after HINMAN (1935a), Chart I.

B. *W. bancrofti* in man, after WARRINGTON YORKE and BLACKLOCK (1917) Table 1.

with *D. immitis* is more important than they see ; their results from concentrated fouadin are given in enough detail to be of use here.

Dogs to which the drug was given every other day lived through a full course of treatment ; from the blood of all of them microfilariae disappeared for good ; all were killed from 27 to 221 days after treatment ; all then had living female worms in the heart ; and all these worms had been sterilized, their uteri being always found empty ; the worms had become spent and remained so. There was in effect a series of controls ; ten dogs

were given daily injections of the drug, microfilariae disappeared from the blood of all in 3 to 5 days, but in three dogs the symptoms of poisoning were so serious that treatment was stopped. Only in these three did microfilariae reappear in the blood—after 11, 11 and 126 days.* One was treated again and microfilariae again disappeared, this time for good. When these ten dogs died or were killed, there were living worms in all, but only in those two in which treatment or re-treatment had failed to clear the blood of microfilariae were microfilariae present in the uteri of female worms : in all other dogs female worms though living had empty uteri.

Fouadin did not kill the larvae after they got into the blood, it damaged the ovaries of the mother worm so that they were not even formed ; it is a sterilizer† not a microfilaricide. JOHNSTONE'S experiments (1936) must not be quoted against this.

In all his four dogs microfilariae left the blood under this treatment ; three dogs died and when then examined worms were still living in two, but no mention is made of the state of the reproductive organs in any female worm ; the fourth dog showed microfilariae again after 118 days, so that, if re-infection can be excluded, this, taken with their reappearance after 128 days in the experiments of the Chinese writers, perhaps hints that sterilization by fouadin is temporary. If that is not accepted, must not the logical conclusion be that *D. immitis* passes young once in 4 months ? But that does not go with the belief that their reproductive life is so short that worms are commonly too young or too old to bear offspring.

Temporary sterilization may be part of the explanation of the work of NOC (1923) on *W. bancrofti* in which intramuscular injections of amino-arsenophenol caused disappearance of living microfilariae for a month only. If some drug can be found which permanently sterilizes *W. bancrofti* without risk, or perhaps without serious risk, to the host it has great possibilities even if not vermidical ; for if the evidence given has been fairly set out, microfilariae take their place beside the silica and quinine of the Boston workers as efficient producers of elephantiasis and are the essential causes in this infection of damage to body and hurt to mind ; so that if their formation can be stopped more safely than the mother worm can be killed, much of the great economic loss and mental distress caused by the infection will have gone. Though not ideal, this would be a great advance in tropical medicine.

(*ii*) *The Significance of* Mf. bancrofti *by Day in the Lungs and Great Vessels of Man.*

It is necessary to realize what confirmed MANSON (1898) in the belief that " during their diurnal absence from the peripheral circulation the microfilariae retire principally to the larger arteries and to the lungs, where during the day they may be found in enormous numbers."

*The writers suggest that there may have been reinfection in the last.

†The procreative state of the male worms is not noted, but if it had been on them only that that fouadin acted, the expectation would be that the ovaries would have gone on producing eggs and that the uteri would have been full of these, unfertilized and undeveloped.

A man with marked and regular nocturnal periodicity killed himself with hydrocyanic acid at 8.30 a.m., and at autopsy 6 hours later microfilariae were crowded in the lungs and " vast numbers were found in smears from the inner surface of the carotid arteries " (MANSON, 1900). Of 7,844 microfilariae counted, the average numbers per slide were : lungs 675, carotid artery 612, heart muscle 122, brachial venae comites 28, spleen 1, liver 2/3, bone marrow 0. MANSON added, " The preparations afforded no explanation as to how microfilariae continue to maintain their position in the blood current, or as to the forces determining their peculiar distribution."

Microfilariae do not maintain their position in the blood current by day and the forces which determine this peculiar distribution come into action after the host's death ; for, as BAINBRIDGE (1906) later showed, lymph may flow freely from the thoracic duct after death in certain conditions, and one of these, as DRINKER proved (LANE, 1935) is death from cyanide. DRINKER tested the point on two dogs, canulating their thoracic ducts and then killing them by intravenous injections of potassium cyanide ; after the heart stopped the lymph flow went on, in one for 33 minutes and in the other for 97·5 minutes and in both it was markedly increased after death (Fig. 4). It may not be taken as true that these numbers mark the greatest lymph flow which may take place after death from cyanide. It follows that the finding of microfilariae in lungs and great vessels after death is no proof that they were there during life ; and when death has taken place from a poison which causes a great postmortem flow of lymph any such assumption has become doubly inadmissible. If in the man, whose examination confirmed MANSON in his view, microfilariae had been present in the filarial lymph plexus (which had taken the place of the thoracic duct) at any time during the great postmortem and post-cyanide lymph flow which we must now conclude there was, they would have been carried by it to those very positions in which MANSON found them. In persisting objection to this conclusion LOW holds that microfilariae are most numerous in the thoracic blood vessels because these are their " seat of selection." He cites a Barbados blackbird in which these were twenty times as many in heart blood directly after death as in peripheral blood just before ; his St. Kitts case who died at 10 a.m. and microfilariae were at autopsy found only in lung blood ; and another man in whom *Mf. perstans* was predominantly so in the blood of the lungs, heart and great vessels. In the first we are without knowledge of the habitats of the worms, whether in blood or lymph vessels, the possible periodicity of the microfilariae and its timing if there was, or the effects that manipulations had on microfilarial distribution ; in the second and third, microfilariae were found just where the normal postmortem flow of lymph would have taken them had they been present in it during that flow. The real point is this. Is it possible for microfilariae to hold their place in a blood current many times greater than their own observed rate of progress (for in O'CONNOR'S sections they had been out in the full stream if their position during life was that existing after death) without any known means of doing so—because such is their wish ? For it surely comes to that. MANSON'S conclusions, based on the imperfect knowledge

of the time and backed by his great reputation, held back for some 40 years further research into the cause of microfilarial periodicity in Bancroftian filariasis.

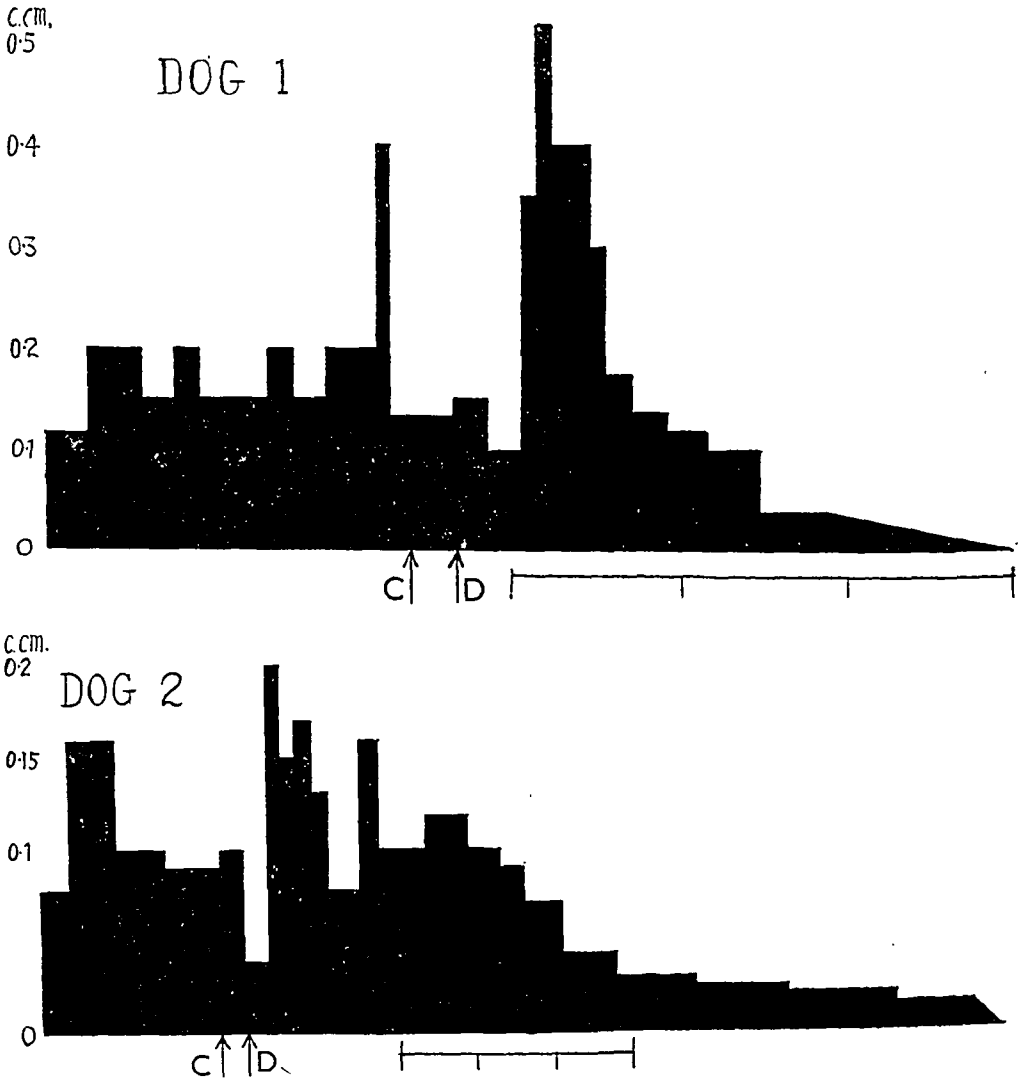


FIG. 4.—Postmortem flow of lymph in two dogs after death from intravenous injection of 1 per cent. K C N solution at the rate of 1 c.cm. per kg.

Experiment done under Professor DRINKER of Harvard University; charts made from the protocols generously given me by him.

C. Injection of cyanide. D. Heart stopped beating.

Scales: 30 minutes, 10-minute intervals shown.

2. THE CAUSE OF THE FALL OF THE MICROFILARIAL TIDE.

As a possible explanation of the fall of a microfilarial tide whose rise was caused by parturition of female worms, I quoted (LANE, 1929) BAHR'S experience (1912) that at body temperature *Mf. bancrofti* does not live outside man's body for more than 12 hours. It is now clear that BAHR'S experience was incomplete. Microfilariae live longer than this at body temperature. If then the fall of the tide is caused by their death, the body is active in their killing; and since it is the reticulo-endothelium which is thus active before they get into the blood, it is the probable agent in any killing afterwards. This idea will now be considered from two aspects. (*i*) How does it fit in with knowledge of the system's working? (*ii*) Is there visual evidence of such action?

(*i*) *Facts explained by Blockage of the System.*

By injection of microfilariae into two series of animals, one with a blocked and one with an unblocked system, there is hope of a biological test of the place the system has in attacking helminths in the tissues—keeping in mind the three reservations about blockage. This has never been done, but the best explanation of observations next considered seems to be such blockage—natural, or experimental but unintentional.

(*A*) *Effects of Injections of Microfilariae into Blood Vessels of an Optimum Host.*

(*a*) Injections of *Mf. immitis* into a dog.

HINMAN, FAUST and DE BAKEY (1934) made at 5 p.m. a mutual exchange of about a third of the blood of two dogs of nearly equal weight. At that hour Dog A had 27,475 *Mf. immitis* to the c.cm. of blood; Dog B, which had none, received 3,846,500 (750 to the gramme of body weight or 9,000 to the c.cm. of blood volume*) and of these 3,538,780 or 92 per cent. were never seen again; the reasonable conclusion is that the unhypertrophied reticulo-endothelial system of Dog B was blocked by these huge numbers, so that 307,720 survivors were able to live for a time in the new host as easily as on a slide, there being no mobile natural enemies to attack them in either case. But if an unhypertrophied reticulo-endothelium could kill these great numbers in this short time, it is unlikely that the many microfilariae in the circulating blood at low tide in a naturally infected dog (in which the system has had time for, and presumably has undergone, hypertrophy) are there because the system is always blocked, but likely that the rise and imperfect fall of the tide are due to imperfectly timed parturition.

There was in these dogs a reversed periodicity on which the writers do not comment.

On the day on which the exchange of blood was later made (at 5 p.m.), the numbers of microfilariae in the blood of Dog A at 3 a.m. and 9 a.m. had been 47,000 and 17,000; next day at those hours they were 9,425 and 13,750. In Dog B the lowest and highest numbers on the day after the exchange were 175 at 3 a.m. and 425 at 11 a.m., though in Dog A the numbers had been at their highest at 3 a.m. and near their lowest at 11 a.m.

*WITTS (1932) puts the blood volume of a man as 4,000 to 8,000 c.cm., or 1/17 to 1/9 of body weight if this were 70 kg. In calculations in this paper the fraction is taken as 1/12 in man and beast, and numbers of microfilariae are given per gramme of body weight as some measure of the size of the reticulo-endothelial system, and per c.cm. of blood as some measure of dilution in this.

If the blood was well sampled, the blood exchange had a great effect on the microfilarial periodicity whatever its mechanism.

Again the microfilarial numbers in Dog A did not get back to normal for 2 weeks and these writers' comment is this :—

" Daily production and destruction of microfilariae would have returned the count to normal within 24 hours." No. Only if these huge losses and replacements of blood left unaffected the dogs, and so the worms of which the dogs are the sole environment. Of the bodily state of the dogs or on any change of the hours they slept the report is silent ; of the disturbed condition of the parasites as seen in their bewildered periodicity there is proof enough ; and for other worms, hookworms for example, the rate of oviposition may be greatly altered by the state of their only environment, the host. The conclusion quoted above holds good only if temporary removal of a third of its blood and its replacement later by blood from a different animal will certainly leave quite unaffected the animal, its blood and the worms that live in its blood. On available evidence we have no right to that assumption.

All the published facts in this *Dirofilaria* experiment have natural explanations in a blocking of an unhypertrophied reticulo-endothelial system and in other disturbances of delicate physiological processes in host animals of whose effects on other parasites we have long known.

(*b*) Injections of *Mf. bancrofti* into man.

Important tests have been made by MURGATROYD, KNOTT and RAO.

MURGATROYD (1933) injected 720,000 microfilariae into man (say 151 to the c.cm. of blood and 10 to the gramme of body weight) ; none were seen again, but they had been 2 hours outside the body and some " showed signs of commencing ecdysis," and this, he felt, may or may not have influenced results.

KNOTT (1935) in the Virgin Islands, where Bancroftian filariasis is endemic, injected microfilariae into eight persons, here numbered 1 to 8. Before injection he found no microfilariae in Nos. 1 to 6, but they were present in Nos. 7 and 8. Into Nos. 1, 2 and 3, in whom he saw no physical signs of infection, he injected respectively 625,000, 1,120,000 and 870,000 microfilariae (say 114, 186 and 145 to the c.cm. of blood and 9, 16 and 12 to the gramme) ; in two, none were seen after the first few minutes, in the third there were some in two films made 48 hours later. Nos. 4, 5 and 6 had physical signs ; No. 4 with enlarged inguinal and subinguinal lymph nodes, orchitis and funiculitis, had 850,000 microfilariae injected (say 141 to the c.cm. and 12 to the gramme) and all disappeared at once ; No. 5 had the same signs and elephantiasis of the arm and leg, had 875,000 microfilariae injected (say 145 to the c.cm. and 12 to the gramme), 20 minutes later 12 were present to the c.cm., and 2½ days later three unsheathed ones in 10 c.cm. ; No. 6 had (when examined later) hydroceles half way down the thigh, was given an injection of 700,000 microfilariae (say 116 to the c.cm. and 10 to the gramme), microfilariae were seen for about a week in average numbers of 21·8 to the c.cm., and there was one in 10 c.cm. of blood on Day 14. Those persons with microfilariae had blood drawn at night, kept at room temperature (84° F. : 28·9° C.) till mid-day and reinjected* ; in No. 7, 2,000,000 microfilariae (say 333 to the c.cm. and 29 to the gramme) were reinjected at a time when the blood had 245 to the c.cm., and in five samples taken within the next hour the mean number was 258 ; No. 8 had 900,000 (say 150 to the c.cm. and 13 to the gramme) reinjected when the blood had 45 to the c.cm., after 10 minutes the rate was 135 and after 2½ hours 35 ; in these persons 2,000,000 and 900,000 microfilariae quickly disappeared.

SUNDAR RAO (1936) injected citrated blood and 120,000 microfilariae (say 22 to the c.cm. and 2 to the gramme) into a vein of a volunteer of the same blood group ; none were seen again in many examinations.

*KNOTT then seems to have felt that MURGATROYD was over-cautious in hestitating to draw conclusions, because of a delay even of 2 hours before reinjection of blood.

KNOTT's injections were made in an endemic area, and absence of microfilariae in the blood is no proof of absence of infection, indeed in this small series a later examination showed hydroceles reaching half way down the thighs in a man who had been thought at the time of injection to be free from all signs of filariasis. In fact, one cannot be reasonably sure that any of the persons was free from infection and so presumably had an unhypertrophied reticulo-endothelial system. In an endemic area it is quite impossible for there to be that essential control of single factors (LANE, 1927) which may be expected in a non-endemic area, and MURGATROYD's experiment is the only one reported outside an endemic area. The reasonable explanation of KNOTT's experiments is that, in all but one, the reticulo-endothelial system was able to deal with injected microfilariae, in No. 6 it became temporarily blocked ; of SUNDAR RAO's that the reticulo-endothelial system was not blocked.

(*B*) *Injections of Microfilariae into a Non-optimum Host.*

Mf. bancrofti in citrated human blood was injected by LOW, MANSON-BAHR and WALTERS (1934) into the hearts of two guineapigs weighing 150 grammes each ; taking their blood volume as 12·5 c.cm. and making allowance for the quantity of blood injected they were given microfilariae at the rate of about 19,000 and 10,300 to the c.cm. or 1,700 and 900 to the gramme and these disappeared at once from the circulation because, so it was held, they were dissolved in an incompatible blood ; no test was made to see if they did in fact dissolve in guineapig's blood *in vitro*. On such uncontrolled reasoning rabbit's blood should be equally incompatible and solvent. for when SUNDAR RAO (1936) injected into these animals human blood containing *Mf. bancrofti* in unstated numbers, none were seen again ; yet I have as controls slides made by O'CONNOR of the organs of rabbits which had had injections of such blood, and in them the microfilariae are obvious ; there was in the guineapigs an immediate removal from the circulation, not a solution. A disappearance of this sort is common equally to optimum and non-optimum hosts and it is not permissible, without a test, to put it down to solution in incompatible blood in the second group, seeing that it certainly is not so caused in the first.

(*ii*) *Microfilarial Destruction Causes no Protein Shock.*

Of microfilarial death as a cause of the fall of the microfilarial tide LOW, MANSON-BAHR and WALTERS (1933) say " A massive destruction such as that postulated would have to be sudden and swift, and the liberation of this amount of foreign protein, one would imagine, would be liable to produce considerable reaction in the human body—a species of protein shock." Further knowledge has shown that destruction of microfilariae does not cause protein shock.

When HINMAN, FAUST and DE BAKEY injected 3,846,500 of them into a dog with immediate disappearance of 3,538,780 there was no protein shock ; when MURGATROYD injected 720,000 into man with their immediate disappearance, there was none ; when KNOTT injected 625,000, 1,120,000, 870,000, 850,000, 875,000, 700,000, 2,000,000 and 900,000 into man with their immediate disappearance in every case but one there was none. Even when adult *D. immitis* was killed by fouadin, JOHNSTONE (1936) says of work by WRIGHT and UNDERWOOD, " They found that the killed worms were eventually absorbed in the parenchyma of the lungs and in most cases the host could take care of the foreign protein of the dead worms with no marked reactions " ; and JOHNSTONE himself with the possibility in mind makes no mention of protein shock in the four dogs he so treated. Further this objection ignores what takes place in every natural infection, for as had been made clear there is destruction of microfilariae before they get into the blood. It must be

taken as great, for O'CONNOR's uncontradicted work shows that a female worm empties her uteri daily ; it must be long-lasting if the considerations put forward as to the latent period are right. Yet no protein shock has been reported with all this.

There seems no reported instance of protein shock from massive destruction of microfilariae by the body before or after they have got into the blood.

(iii) The Mechanism of the Fall of the Microfilarial Tide and its Site.

Microfilariae are destroyed by reticulo-endothelium before they get into the blood, and the outcome of much experimental work is what it would be if the same system destroyed them after they have ; so this question arises. Is there any visual evidence that destruction is the cause of the fall of the microfilarial tide. There is little ; yet convincing evidence is apt to be little when not looked for. But first it is well to go over several conditions helpful or needful for the drawing of safe conclusions when testing the supposition under consideration.

(1) A double mechanism will be in action as the cause of the fall of the microfilarial tide, namely, physical arrest and chemical solution in that order, the former being shown in SUNDAR RAO'S experiment and O'CONNOR'S slides from the rabbit ; and both being seen in DUDGEON and GOADBY'S experience (1931) with *Staphylococcus aureus* injected into the circulating blood, when these were taken out of this within 5 minutes by polymorphs in the lungs but finally destroyed by Kupfer cells in the liver. (2) There will be no great enlargement of the tissue that destroys those microfilariae which have got into the circulating blood. It has been noted that before they do so they are destroyed in lymph nodes ; it is true that these last enlarge, but equally true that part, at least, of the not great enlargement is caused by varicosity of lymph vessels and not by increase of active tissue ; reticulo-endothelium, then, destroys microfilariae very efficiently before they get into the blood and presumably will be as efficient after they have, so we must not look for bulky hypertrophy as a guide to any part or tissue concerned. (3) Any solution of microfilariae by the system must be rapid, if not they would pile up and have been detected years ago. (4) The most promising sites to search for this destruction are those where there is most of this tissue. (5) In a periodic infection the destruction will presumably have a periodicity, perhaps as clear as that reported by O'CONNOR for the worm's parturition.

Evidence for the last should be sought in persons in whom there are many microfilariae in the blood at high tide and few or none at low, where infection is heavy and periodicity definite. As to the first, a heavy infection will lessen labour of searching slides particularly as distribution of microfilariae in an organ may be patchy. As to the second, it is useless to look for periodic daily destruction of all microfilariae if the blood does not show periodicity ; moreover, the presence of this must be established for each individual tested for it seems that in districts where periodicity is the rule, the usual complete fall of the tide may fail where infection is heavy—as would be the case were there some blocking of the reticulo-endothelial system. Before, then, full proof can be got of a daily

cycle of microfilarial destruction in periodic Bancroftian filariasis, it must be established that periodicity was present in all persons used for tests, these must cover many hours of the twenty-four, and they must be made in conditions as near to those of control of single factors as can be managed.

It has been noted that the most promising sites to search for a daily cycle of microfilarial destruction are those rich in mobile cells of the reticulo-endothelial system, that is to say lymph nodes, connective tissue, bone marrow, liver and spleen. Since microfilariae come to *lymph nodes* by both lymph and blood and since the adults may be widespread in the body (O'CONNOR, GOLDEN and AUCHINCLOSS, 1930) it is, at present at least, difficult to say that any particular microfilaria has got to a node by blood. For the lymph-bathed *connective and elephantoid tissues* there is the same difficulty, and though there would be poetic justice did elephantiasis which the larvae have caused prove a means for their readier destruction, it is well to keep poetry and helminthology distinct. *Bone marrow* has, I believe, never been studied with this possibility in view; it should be. The *liver* and *spleen* have been examined by O'CONNOR* in two persons, with the results now considered.

S. Murdered "about 1 a.m." Few microfilariae were seen in blood vessels cut through in sections, the few present in the spleen pulp were not degenerate.

H. Died at 1.15 a.m. from aortic disease having been in hospital for 3 weeks with dyspnoea, oedema of legs, ascites and restlessness; he slept only for about an hour at a time, was semi-stuporous for the last week and cyanotic at death. Many microfilariae were present in blood vessels cut through in microscopic sections. Except for a healthy fringe of cells round hepatic lobules, the liver cells were degenerate while among them were most of the microfilariae seen in that organ. The structure of the microfilariae seen in the lungs was normal or, perhaps in some instances, only nearly normal. On the other hand, many of those in the spleen and liver were markedly changed, with nuclei scattered, poorly stained or ring shaped, and the whole body swollen with local bulgings and kinks; there were no cell collections round them in either organ; the probability has, I think, to be kept in mind that some at least of these degenerate microfilariae in the liver may have drifted there in the splenic and portal veins and been filtered out, as AUGUSTINE (1937b) concludes they may be in the frog.

In these two examinations there was not, and could not have been, control of single factors, and in fact they differed in many particulars.† For instance, while the presumption is that the microfilarial tide was normally timed in *S.*, in *H.*'s abnormal condition this is at least doubtful; and without certainty as to this, no knowledge can be had that the normal curves of the fall of the microfilarial tide and of microfilarial destruction are alike. Yet the conditions found in *H.* are significant in two respects. Firstly, the appearances of the degenerating microfilariae are the same as AUGUSTINE (1937b) reports from the frog, and secondly this very marked microfilarial degeneration was present in *H.* in the

*The generous gifts by Professor F. W. O'CONNOR of Columbia University, New York, of sections from these autopsies and of the photographs which illustrate this paper, and his kindness in commenting on the typescript, lay me under a great obligation.

†For this reason I hesitate at this time to put forward as unquestionable confirmation of periodic microfilarial destruction the findings, especially in Postmortem I, of J. ANDERSON (1924, Res. Mem. Lond. Sch. trop. Med. 7; Pathology); for there was there the complication of septicaemia.

spleen and liver but not in the lungs. These two sets of facts seem definitely to put aside postmortem change as the cause of the degeneration and to form an urgent pointer to the need for close examination of these organs (and of the marrow) after deaths*, covering many hours of the twenty-four, in the search for evidence of a periodicity of microfilarial destruction as the ultimate cause (apart that is from immediate arrest) of the fall of the microfilarial tide. Though the evidence here put forward that this is so does not amount to proof, it shows that there is at times in man's body that massive destruction of microfilariae which would be expected were this the cause of the fall of the microfilarial tide, and it points reasonably to an unblocked and probably hypertrophied reticulo-endothelial system as the agent here in action.

In 1929, I gave reasons for the belief that MANSON's view of the cause of the microfilarial tide in Bancroftian filariasis could not be correct. There followed the expected refusal to accept a refutation of MANSON's opinion. Yet since then all new knowledge, so I judge, has added evidence for the truth of my view that timed parturition is the cause of the rise of the microfilarial tide, while during the intervening 8 years active critics have published no report of independent and open-minded work done on O'CONNOR's great scale (or on any other) to confirm or confute his anatomical findings. His later examinations begin to shed light on the cause of the fall of the microfilarial tide, and it will be most interesting to see how much time will pass before adequate independent work is done to focus that light. Is the average lag in such cases 10, or is it 20, years ?

IV.—SUMMARY.

In Bancroftian filariasis the rise and the fall of the microfilarial tide are caused by different mechanisms. There is now anatomical evidence, furnished by O'CONNOR, that the rise is caused by timed parturition of female worms, and here the significance of spent worms has been imperfectly grasped either as a normal daily event or as a result of their sterilization by drugs. The fall of the tide is caused by the mobile cells of the reticulo-endothelial system, for which statement the line of argument runs thus. When helminths enter the tissues of a host animal, it is these very cells which collect and attack them actively. For Bancroftian filariasis their presence was reported years ago, but their fundamental importance has been missed. They destroy microfilariae in lymph nodes before they get into the blood, and by their accumulation and their development to the end cell of the series, the fibroblast, they cause lymphatic obstruction ; the lymph conditions then become those of a culture medium which may be four million times better suited to the haemolytic streptococcus than is normal lymph, and when infection with this bacterium has set in they become still more favourable for growth of fibroblasts and increase of elephantiasis.

*It may prove possible in certain abdominal operations to use for this investigation a " simple and safe method of liver biopsy," as was done by CAMPBELL (1936), on the advice of Professor H. H. LOUCKS of Peiping, to determine the relationship of schistosomiasis to Banti's syndrome.

When microfilariae have got, or been put, into the blood, many events are those which would take place were the reticulo-endothelial cells active in their destruction, the others those which would be seen were the system blocked by them ; these are taken up in detail, and it is shown, first that protein shock is not caused when injected microfilariae disappear from the circulation at once and for good, even by the million, and second that the remarkable post-mortem flow of lymph, which is caused by cyanide poisoning, would have taken any microfilariae in the post thoracic lymphatics to just those positions in the great arteries in which MANSON found them in his now classical case; that he found them there after death is no evidence that they were there during the man's life.

Of anatomical evidence that destruction of microfilariae causes the fall of the microfilarial tide there is little—not surprising since almost no one has looked for it.. But in one of two cases sectioned by O'CONNOR there had been that massive destruction of microfilariae in spleen and liver which would be expected did the reticulo-endothelial system get rid of them daily. This finding calls for investigation of these organs, and of the bone marrow, after death has taken place at different hours in persons in whom periodicity is known to have been maintained to the end, in order to see what relationship there is between the microfilarial tide and the extent of destruction of microfilariae. Limited biopsy may help here. There is the possibility of great benefit to those with this infection were there found a drug which would safely sterilize, even if it could not kill, all female worms. A detailed and unbiased examination of the whole subject of the cause of periodicity is urgently needed.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

STUDIES IN TRYPANOSOMIASIS.

I.—THE ELECTRIC CHARGE OF TRYPANOSOMES IN TSETSE FLIES.

BY

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AND

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In a previous communication (BROOM, BROWN and HOARE, 1936) it was shown that trypanosomes in the blood of infected animals can carry either a positive or a negative electric charge. A simple test for the determination of the sign of the charge, capable of being used in the field, was described. In the present paper the test has been modified for the study of the charge of trypanosomes in the tsetse fly.

*This investigation has been made possible by the kindness of Dr. J. F. CORSON who has sent us a regular supply of pupae of *Glossina morsitans* from Tanganyika.

We have used in these experiments a strain of *Trypanosoma brucei* U.D. which, as we noted in our previous paper, was supplied to us by Prof. WARRINGTON YORKE. While this work was in progress Professor YORKE informed us that this strain had lost its transmissibility by the fly. Our experiments, as far as they go, are in accordance with this. We have found no evidence of salivary gland infection in flies which have shown heavy gut infection up to 39 days after the infective feed. Altogether we have dissected 987 flies at intervals of 11 to 39 days after the infective feed. Of these, 58 showed gut infection but there was no salivary gland infection. The fact that the cyclic development in the fly was incomplete has somewhat limited the scope of the present investigation as we were thereby unable to study the electric charge of the trypanosome throughout the entire cycle. We have, however, obtained certain data on the electric charge of the organism during its development in the gut of the fly.

TECHNIQUE.

A tube containing three flies is applied to the shaven thorax* of a mouse showing a pure infection with either positively or negatively charged trypanosomes, and those flies which feed are separated and transferred to a larger cylinder. The flies are subsequently given the opportunity daily of feeding on a chicken and are finally dissected and examined after stated intervals. Separate chickens are reserved for the nutritive feed of flies originally infected with positively and negatively charged trypanosomes respectively.

The strains in these experiments were obtained by infecting mice with single trypanosomes of known charge.

THE DETERMINATION OF THE SIGN OF THE CHARGE IN THE FLY.

In our previous paper we described a simple test for the determination of the sign of the electric charge of trypanosomes in the blood of infected animals. It consists of diluting a small quantity of blood containing trypanosomes with glucose-saline (one volume of normal saline and nine volumes of 4 per cent. glucose in distilled water). The mixture is allowed to stand for a few minutes and then examined under a microscope. Positively charged trypanosomes will be seen to be firmly adherent to the red corpuscles while the negatively charged are free.

When studying positively charged trypanosomes at short intervals after ingestion by the fly we came to the conclusion that our previous technique

*Flies were originally fed on the abdomen of the mouse but this proved unsatisfactory, as in certain cases the proboscis of the fly must have penetrated the alimentary canal of the mouse. In one fly, dissected 3 hours after feeding, various flagellates, *Trichomonas*, *Giardia* and *Hexamita* were found in the gut contents. Preparations showing these intestinal flagellates from the gut of the fly were demonstrated by Dr. WENYON at a laboratory meeting of this society (see this number of TRANSACTIONS, page 7).

would have to be modified. We found that these trypanosomes, presumably still positively charged, behaved in the salt-concentration test as though they were negatively charged.

The following experiment shows that this effect is due to the action of the secretions present in the fly.

Three flies were fed on normal uninfected mice and dissected 24 hours later. The intestinal contents together with the gut were broken up in 0·5 c.c. of glucose-saline and this ingested red cell suspension was used as shown later. As a control, blood from a normal mouse was taken directly into citrated saline, the cells were washed twice and resuspended in glucose-saline. This provides the normal red cell suspension. The blood of a mouse infected with positively charged *T. brucei* was taken into citrated saline and centrifuged slowly to throw down the red cells. The supernatant fluid containing the trypanosomes was then centrifuged rapidly and the deposit of trypanosomes resuspended in glucose-saline.

Thus we have three reagents, all suspended in glucose-saline :—

(1) Ingested red cell suspension. (2) Normal red cell suspension. (3) Suspension of positively charged trypanosomes.

When (2) and (3) were mixed the trypanosomes were seen to be firmly adherent to the red cells, whereas when 1 and 3 were mixed, no such adhesion took place. From this it is obvious that the ingested red cell suspension has lost its power of giving adhesion with positively charged trypanosomes.

In a second experiment the ingested red cell suspension was centrifuged and the supernatant fluid retained. The cells were washed and resuspended in glucose-saline. Washed, positively charged trypanosomes were added to these cells and were now seen to show strong adhesion. The supernatant fluid mentioned above, when added to normal red cells and positively charged trypanosomes, entirely prevented adhesion taking place.

These two experiments show that the inhibition of adhesion which occurs when ingested blood is used is caused by the action of some constituent present in the gut of the fly. We came to the conclusion that this constituent is the secretion of the salivary glands which mixes with the ingested blood. We found that isolated salivary glands macerated in glucose-saline completely inhibited adhesion between normal red cells and positively charged trypanosomes. This appears to be in accordance with the finding of LESTER and LLOYD (1928) who showed that the proventriculus and first third of the mesenteron are themselves inert in relation to coagulation of blood, but as removed from the normal fly they contain the anticoagulin which is derived from the salivary secretion.

To overcome this interference we finally adopted the following procedure which makes the test applicable to the study of the electric charge of the trypanosome in the gut of the fly. The gut is macerated in about 10 c.c. of normal saline and the suspension is centrifuged slowly to throw down the coarser particles and red corpuscles, leaving the trypanosomes in the supernatant fluid.

This is pipetted into a clean tube and centrifuged rapidly to precipitate all the trypanosomes. The supernatant fluid is poured off and the sediment resuspended in about 15 c.c. glucose-saline. This suspension is again centrifuged rapidly and the supernatant fluid drained off. To the minute quantity of glucose-saline containing trypanosomes which remains in the tube is added a small drop of fresh normal mouse blood. This is more satisfactory than relying on irregular quantities of ingested red cells. The contents of the tube are then thoroughly mixed and after standing for a few minutes are examined with the microscope. As in the previous test positively charged trypanosomes are firmly adherent to the red cells while negatively charged trypanosomes are free.

The Sign of the Charge of the Trypanosome in the Fly.

In the case of flies fed on mice infected with negatively charged trypanosomes, the sign of the charge in the fly invariably remained negative.

As regards flies fed on mice with positively charged trypanosomes the position is somewhat more complicated. Up to 12 hours in the fly the trypanosomes still carried a positive charge. Thereafter an increasing percentage of trypanosomes became negatively charged. After 48 hours only very few positively charged trypanosomes remained and at 72 hours only negatively charged trypanosomes could be found. When, however, mice were injected with the gut contents of these flies, originally fed on positively charged trypanosomes, any infections which resulted were always due to positively charged trypanosomes. There would appear to be two possible explanations of this: (1) The positively charged trypanosomes which had become negative in the fly reappear in the mouse as positively charged. (2) The infection in the mouse is due to the survival of a few positively charged trypanosomes which, owing to their small numbers, were not detected when examining the gut contents of the fly.

We hoped to be able to prove this point by injecting mice with single trypanosomes whose charge we had determined, but after 48 hours the infection in the fly becomes so scanty that this has proved impossible. This is due to the fact that in isolating single trypanosomes from suspensions containing red cells it is necessary to dilute until there are very few cells in the droplet, otherwise a single trypanosome might be obscured. When only very few trypanosomes are present originally this procedure is impracticable.

Period of Infectivity in the Fly.

The length of time that trypanosomes remain infective in the gut of a fly is well known, but we thought that it would be interesting to test whether there was any difference in this respect between positively and negatively charged variants. As is shown in the table, infection takes place in both cases up to 72 hours, but not at 96 hours.

PERIOD OF INFECTIVITY IN THE GUT OF THE FLY AND CHARGE OF SUBSEQUENT INFECTION.

Period in Fly.	Number of Mice Inoculated.	Number showing Infection.	Charge of Trypanosome.
TRYPANOSOMES ORIGINALLY POSITIVELY CHARGED.			
24 hours and less	79	53	Positive
48 hours	21	5	"
72 "	33	8	"
96 "	12	0	—
TRYPANOSOMES ORIGINALLY NEGATIVELY CHARGED.			
24 hours and less	13	13	Negative
48 hours	35	15	"
72 "	18	1	"
96 "	21	0	—

CHARGE OF DEVELOPMENTAL FORMS IN THE FLY.

In a certain number of instances, we have been able to determine the charge of trypanosomes present in the gut of the fly at intervals of 17 to 33 days after the infective feed. Altogether twenty flies fed on positively charged trypanosomes from 17 to 28 days previously, and thirteen flies similarly fed on negatively charged trypanosomes from 17 to 33 days previously were examined. Irrespective of the original charge all the trypanosomes were found to be negatively charged. As we have already noted, no salivary gland infections were produced, but thanks to Dr. CORSON having sent us other strains known to be transmissible by fly we hope now to be able to study the entire cycle.

As it is known that infection can occasionally be obtained with the gut forms, we inoculated mice on thirty-six occasions with the intestinal contents of flies dissected at least 3 weeks after the infective feed. In only one instance was infection produced in the mouse. In this case the fly had been fed 22 days previously on positively charged trypanosomes and the infection which appeared in the mouse was due to trypanosomes of the same charge. Unfortunately the trypanosomes were so scanty in the fly that it was impossible to determine their charge.

SUMMARY.

1. The salt-concentration test for the determination of the sign of the charge of trypanosomes has been modified to make it applicable to the study of the charge of the developmental forms in the tsetse fly.

2. Negatively charged trypanosomes in the infective feed remain unchanged in the gut of the fly.

3. Positively charged trypanosomes become negatively charged after approximately 24 hours.

4. Trypanosomes of positive or negative charge retain their infectivity in the fly for the same length of time—namely, about 72 hours.

5. Developmental forms are all negatively charged.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937

STUDIES ON THE INTESTINAL PROTOZOA OF RATS.

I.—RATS AS CARRIERS OF DYSENTERIC ENTAMOEBAE.

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Although the amoebic fauna of rats has been studied by numerous authors, amongst whom may be mentioned WENYON, KOFOID, KESSEL and EPSTEIN, there is still disagreement concerning the normal amoebae of the rat's intestine. Varying descriptions of the amoebae encountered in the rat have been given, and they have been placed in a number of different genera.

LYNCH (1915) was the first to find spontaneous dysenteric amoebiasis in a wild rat in Charleston, U.S.A. The rat had been caught in the negro district of that city, in a house without any drainage system, the basement being used instead of a water-closet by the inhabitants of the house. The author also reported several cases of human amoebic dysentery in this district, apparently of local origin. Caecal lesions, characteristic of amoebic dysentery, were found by him in the naturally infected rat. He identified the amoeba on morphological grounds as *Entamoeba histolytica* Schaudinn. In order to confirm his conclusions, LYNCH fed rats with human faeces, containing cysts, an experiment which was successful in producing infection.

The findings of LYNCH were criticised (CHATTON, 1917; DOBELL, 1919; KESSEL, 1923) on the ground that he had limited himself to a morphological

*Prof. G. W. EPSTEIN died while this work was in progress.

description of the vegetative form and had made no mention of the typical quadrinucleate cysts of *E. histolytica*. Furthermore, it was claimed that he did not study the biology of the amoebae from the rat and had not infected susceptible animals nor attempted to make cultures.

However, 4 years later BRUG's observations in Java corroborated the conclusions of LYNCH while CHIANG (1925) reported the finding in seven white rats of amoebae, having a diameter of 12 to 30μ, which could be readily cultivated in the medium of Boeck and Drbohlav. The author found in preparations stained with Heidenhain's iron haematoxylin that 60 per cent. of the amoebae showed nuclei with a central location of the karyosome, while in the rest of the amoebae the karyosome was slightly eccentric. The cytoplasm contained numerous bacteria. In addition to the trophozoites were found one-, two- and four-nucleate cysts, 10 to 13μ in diameter. The nuclei in these cysts structurally resembled those of the encysted stages of *E. histolytica*. The cysts contained chromatoid bodies varying in size and shape ; in no case however did they have the form of a rod with rounded ends, which is typical of these bodies in *E. histolytica*. The author succeeded in infecting rats with cultures of these amoebae, but the infection produced no apparent clinical symptoms. On the other hand five kittens, out of a total of eight inoculated, became infected and showed symptoms of dysentery. One of the kittens showed during life amoebae containing phagocyted red cells, but no lesions were found in its intestine at autopsy nor were amoebae found in histological sections.

CHIANG succeeded in producing amoebiasis in rats both by feeding them on faecal material from a human cyst-carrier and by injecting them rectally with material from an infected kitten. From these rats he transferred the disease to kittens and produced amoebic dysentery, the amoebae invading the mucous membrane. The author concluded that the results of this investigation incriminate the rat as a possible carrier of *E. histolytica*, the causal agent of amoebic dysentery. He regards the amoebae found by him in rats as a distinct type of *E. histolytica*.

Of the numerous studies on experimental amoebiasis of rodents we consider it necessary to refer to the reports of KESSEL (1923a and b, and 1924) on amoebiasis of rats. This observer studied the normal amoebae of the rat, elaborated a technique for the experimental infection of these animals and for obtaining them amoeba-free, determined the degree of their susceptibility to amoebic infection and the duration of such an infection when once established. He succeeded in infecting rats with five species of human intestinal amoebae. The conclusion which he finally draws is : " The findings of this investigation afford evidence in favour of the morphological constancy of species of parasitic amoebae, under conditions of change of host, and of the possibility of transfer of one species of parasitic amoeba from one species of host to another. Consequently heavy suspicion is thrown on the rat and mouse as possible carriers of the causative organisms of human amoebiasis."

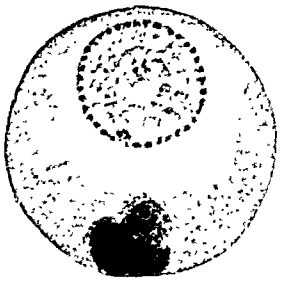


FIG. 1



FIG. 2.

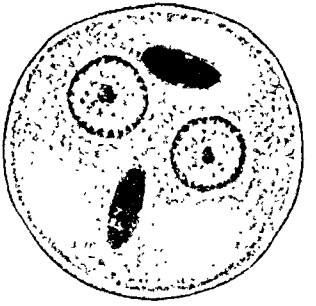


FIG. 3.

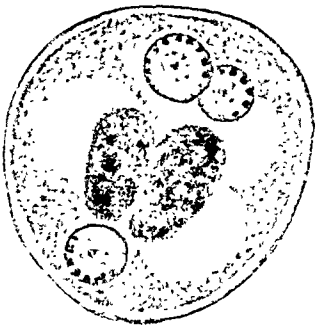


FIG. 4.

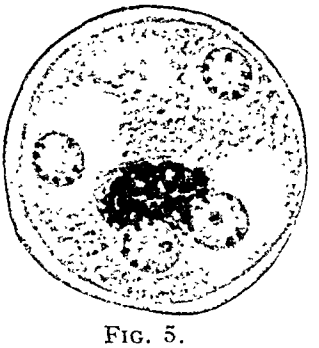


FIG. 5.

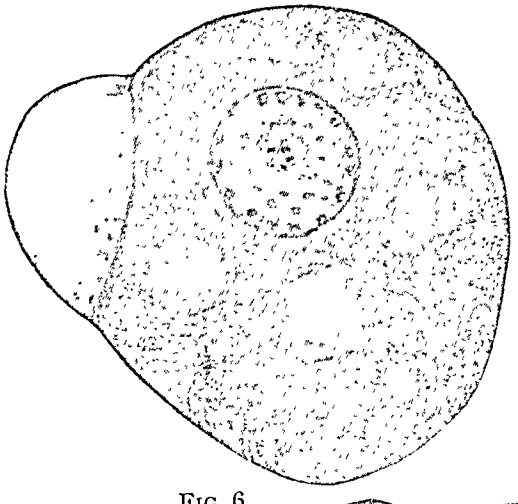


FIG. 6.

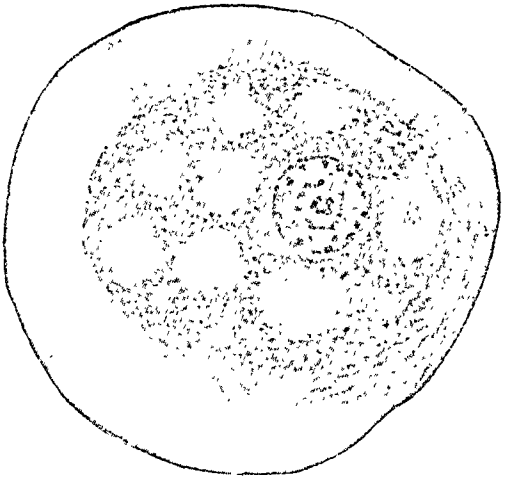


FIG. 7.

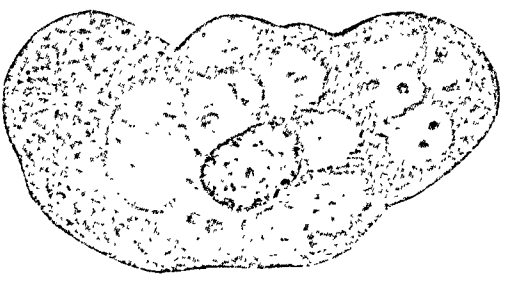


FIG. 8.

FIGS. 1–5.—Cysts (with 1, 2 and 4 nuclei, and chromatoid bodies) of *Entamoeba histolytica* from naturally infected rat.

FIGS. 6–8.—Active forms of *E. histolytica* from naturally infected rat.

To face page 89.

The Discovery of Entamoebae in Moscow Rats.

For the purpose of studying the normal protozoa of rats, we investigated 515 wild rats (*Mus decumanus* = *Rattus norvegicus*) from about thirty separate sources, distributed throughout the ten districts of Moscow, with the result that 6 to 7 per cent. were found to be carriers of various species of *Entamoeba*, among which the *E. muris* type was the prevailing one; certain saprophytic amoebae were also encountered (Epstein, Matevosian, 1926). In addition to the wild rats our material included ninety white laboratory rats. Of these 90 per cent. showed infection with entamoebae of the *E. muris* type. (In Kessel's material 34 per cent. of white laboratory rats harboured amoebae.) Our white rats were obtained from six different sources, and their ages varied greatly, from that of sucklings to old adults. The rats were killed and the intestinal contents examined immediately while fresh. Not less than three stained preparations on cover slips stained with iron haematoxylin were made from each. Cultures of the entamoebae were also made from every positive case in the medium of St. John and Boeck-Drbohlav.

Entamoebae of the *E. muris* type were found both in white laboratory rats and in wild rats.

Apart from amoebae belonging to the genus *Entamoeba*, we encountered much more frequently representatives of the genera *Iodamoeba* and *Endolimax*. Moreover we found in eight wild rats from one source a ciliate, morphologically indistinguishable from *Balantidium coli* of human origin. The description of these findings forms the subject of a separate report.*

In seven wild rats, i.e., in 1·3 per cent. of our total material we found entamoebae which could not be distinguished from *E. histolytica* Schaudinn, 1903, either morphologically, or biologically (Figs. 1–8); they are probably identical with those found by Lynch (1915) and by Chiang (1925) in North America, and regarded by them as a separate variety.

Of these seven wild rats, two had been captured in the grounds of the Moscow Zoological Garden, four in a big house in the central part of the city, and one in a house in which we had found two cases of human amoebic dysentery. As in Lynch's case, the infection bore a focal character, but unlike the case described by Lynch, our rats did not show any symptoms attributable to this infection. Only vegetative forms were found in the rats caught in the Zoological Garden. Many of the amoebae possessed an easily distinguished hyaline ectoplasm; they did not, however, show the characteristic locomotion and the nucleus could only be recognized with difficulty.

In stained preparations the amoebae did not differ in any way from typical *E. histolytica*. Their nuclei (Figs. 6–8) were large, reaching 4·5 to 5μ in diameter with a central karyosome, and poor peripheral chromatin; fine chromatin granules

*In this number of the Transactions p. 93.

often formed a crescent. As regards food inclusions, only bacteria were found. Entamoebae of the *E. histolytica* type were not found in white rats.

Experimental Infection of Kittens.

Cultivation in St. John's medium produced abundant growth of typical motile entamoebae, indistinguishable from *E. histolytica* either by the character of their nuclei and cytoplasm or by the formation of broad hernia-like pseudopodia.

Two kittens (Kittens 3 and 5), inoculated with the second subculture of these amoebae, developed typical amoebic colitis. Numerous entamoebae showing the typical locomotion of *E. histolytica* and large vacuoles with phagocyted red cells were present in their sero-sanguinous excreta. Certain amoebae showed chromatoid inclusions.

From the faeces of these infected kittens, both of which died, the entamoebae were again transferred to artificial media, and a culture was obtained which on the 5th day produced typical quadrinucleate cysts. Acute hyperaemia and punctate haemorrhages of the colon were found at autopsy. In histological preparations entamoebae were seen to have invaded the mucous membrane.

PROTOCOLS.

Protocol 1.—Kitten 3 was inoculated per anum with the culture obtained from Rat 189 on 9.5.35. Infection could not be established either microscopically or culturally till 15.5.35 when the kitten began to suffer from diarrhoea, the faeces being liquid and sero-sanguineous. Microscopical examination showed amoebae with phagocyted red blood cells. The number of stools exceeded thirty during the day. The kitten died the same evening. At autopsy the lungs and liver were seen to be unchanged but the colon was hyperaemic and marked with punctate haemorrhages. In sections of the colon amoebae were found in the superficial layers of the mucosa within the epithelial lining, which showed necrobiotic changes and desquamation in the areas invaded by the entamoebae.

Protocol 2.—Kitten 5 was inoculated per anum on 20.5.35 with culture obtained from Rat 281. On 21.5, 22.5 and 23.5 infection could not be established either clinically, microscopically or culturally. On the 23rd the kitten was inoculated for the second time with the third subculture of the same strain. On 25.5.35 the kitten passed a drop of sero-sanguineous liquid. In this motile amoebae were found. During the following 12 hours the kitten passed fifteen or sixteen liquid stools. On the following day (26.5.35) the kitten had become very thin and it died towards the evening. At autopsy the colon was seen to be markedly hyperaemic and dotted over with punctate haemorrhages; it contained sanguineous mucus in which were found numerous motile amoebae, with phagocyted erythrocytes. Attempts to infect two other kittens (Kittens 6 and 7) with material from Kitten 5 failed. In sections of the colon amoebae were seen to have penetrated deeply into the mucous membrane.

In three of the four positive rats from the second source we found typical, motile amoebae, identical with those found in the rats from the first source. Besides this we found in them numerous cysts with a diameter of 13 or 14μ and indistinguishable from those of *E. histolytica*. They possessed 1 to 4

nuclei and contained the characteristic chromatoid inclusions (Figs. 1–5). In Rat 441 precystic forms were also found.

These amoebae were also cultivated and the cultures proved to be pathogenic for kittens. From the contents of the caecum of one of these, a culture of entamoebae was isolated, the third subculture of which produced typical quadrinucleate cysts, 12 to 14μ in diameter.

Protocol 3.—Kitten 8 was inoculated on 19.6.35 with culture obtained from Rat 441. Motile amoebae with broad hyaline pseudopodia and included red blood corpuscles were found in the faeces of this kitten on the 3rd day. The kitten was sacrificed and at autopsy numerous punctate haemorrhages and small ulcers of the colon were found. In sections entamoebae were found both in the superficial and in the deep layers of the mucosa, while the tissues invaded by the entamoebae showed necrotic changes.

Summary and Discussion.

In addition to saprophytic amoebae, we have found in several wild rats in Moscow entamoebae which resemble very closely *E. histolytica* both in the vegetative and the encysted stages. The cysts were quadrinucleate, had a diameter of 12 to 15μ, and possessed characteristic chromatoid bodies. They differed in no respect whatever from the cysts of the human entamoeba. Cultures showed complete identity with those of typical *E. histolytica* ; and with them kittens were successfully inoculated, the resulting infections being indistinguishable both clinically and pathologically from those produced by human strains of *E. histolytica.*

It must be noted that this is the first time that entamoebae indistinguishable from the human *E. histolytica* have been discovered in wild rats in Europe. The cases had a focal character, for the seven spontaneously infected rats belonged to only three of more than thirty rat colonies investigated.

As in Lynch's case, so in one of our cases in Moscow, the house in which the rats were caught had no drains. A thorough examination of all the inhabitants of the house revealed two cases of amoebic infection in one family (chronic in the father, and acute in his 5-year-old daughter). It was not possible to establish any connection between these cases and the endemic foci of amoebic dysentery in U.S.S.R. (Transcaucasia, Middle Asia and the Far East), or any contact with people who had arrived from such localities. On the other hand there was a very close association with rats. The family had no cupboard and food was left overnight on the table ; the water was kept in an open bucket. Rat excrement was present on the table and in the drawers. Investigation of the intestinal protozoa of the inhabitants showed a close similarity to those of the rats infesting the house. Besides *E. histolytica* described above, we found all the inhabitants of the house and all the examined rats to be carriers of *Giardia, Trichomonas* and *Hymenolepis nana.* The last named is known to infect human beings as well as rats and it is possible that the human intestinal

flagellates may also find these animals to be suitable hosts. From the observations recorded in this paper we consider there is some justification in regarding rats as carriers of the causative agent of amoebic dysentery which may be transmitted from them to human beings.

The introduction of the rat factor into the epidemiology of amoebic dysentery provides one explanation of the so-called autochthonous cases of amoebic dysentery in countries with a moderate or cold climate, i.e., cases in which every connection with the endemic foci of the disease can be excluded, and the origin of which has hitherto often remained far from clear. It must be admitted, however, that the chief transmitter of amoebic dysentery is the human carrier of *E. histolytica*; but the role of rats, though it may be of secondary importance, must be taken into consideration by epidemiologists interested in the spread of amoebiasis.

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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

STUDIES ON THE INTESTINAL PROTOZOA OF RATS.

II.—RATS AS CARRIERS OF *BALANTIDIUM.*

BY

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HISTORICAL.

Only one of the nine species of Infusoria recorded from the human intestine is pathogenic for man : *Balantidium coli* Malmst., 1857. According to LEUCKART, LEEUWENHOEK was the first to discover Infusoria in human faeces in 1722. MALMSTEN found probably the same Infusoria in 1857 in the stool of a patient suffering from severe diarrhoea. Due to its morphological resemblance to *Paramaecium* it was given the name of *Paramaecium coli* syn. *Balantidium coli* Stein, 1862. SOLOVIEV (Tomsk, 1901) was the first to prove that *Balantidium* penetrates into the intestinal wall and to show its aetiological role in certain cases of dysentery. RSCHANITZIN (1925) published a very detailed account of the pathogenesis and the pathology of balantidiasis.

Balantidia are widespread in the animal world. They have been recorded from thirty-three different hosts : (man, monkeys, pigs, wild boars, sheep, horses, bovines, guineapigs, fowls, fish, turtles, frogs, cockroaches, etc.).

The balantidium of the pig is morphologically similar to *B. coli* Malmst., therefore, great epidemiological significance has been ascribed to it. LEUCKART (1861) was the first to report that domestic pigs were carriers of balantidia. BRUMPT in Paris found balantidia in every fifth pig examined. ZHEGALOV in Leningrad found that 25 per cent. of pigs were carriers of this ciliate ; while KAPSHIDZE in Tiflis found it in two-thirds of all the pigs examined. A similar figure (66·25 per cent.) was obtained by PRITZE (1928) from the examination of 400 pigs.

Pigs are only carriers of balantidia, which never provoke in them any disease and do not penetrate the intestinal wall : balantidia may encyst in the pig's intestine. In the case of man they provoke definite lesions, and do not encyst.

The attempts of GRASSI and CALANDRUCCIO, WISING (1885) and ZIEMANN to infect themselves with cysts from the pig were unsuccessful, and this has led to the assumption, that the balantidium of the pig was different from that of man.

In the pig MCDONALD (1922) distinguishes two species which differ in shape, size, and character of the cytostome and of the macronucleus : *B. coli* and *B. suis*. *B. suis* has an elongated shape, the anterior part of its body is wider than that of *B. coli*, and its longer cytostome extends in the direction of the vertical axis ; its macronucleus is sausage-shaped, it is thinner than that of

B. coli, and also occupies a longitudinal position. The length-width ratio of *B. suis* is 1·58 to 2·7 (1·99), that of *B. coli* 1·08 to 1·5 (1·3). HEGNER (1923) considers these distinctions to be only individual variations and not of specific value. In a later work, however, HEGNER (1934) attempts to solve the species problem of *Balantidium* found in the various animals on the basis of measurements of the length, width and surface area of the parasites and of their nuclear apparatus. DOGIEL and GNESDILOFF (1935) consider that size differences and variations of the length-breadth ratio depend on the parasite's nutrition, i.e., on the amount of starch ingested by it. Recently KARIBOV (1936) in Tiflis, after 5 years' study of the problem, expressed the opinion, that *B. coli* Malmst. and the balantidia of the pig are morphologically indistinguishable, thus corroborating the views of WALKER (1913), WENYON (1926) and BRUMPT (1927) on the identity of both parasites and on the pig's role as reservoir of the causative agent of human balantidiasis.

Literary data show that the history of most cases of balantidiasis includes contact with pigs. Some facts, however, remain inexplicable if one accepts wholly the "pig theory" of the origin of human balantidiasis. Thus, for instance, no cases of balantidiasis of local origin have been, so far as I know, described in England, a pig-breeding country, while in Armenia, where there is scarcely any pig-breeding, balantidiasis is of relatively frequent occurrence. TZATURIAN (1935) records twenty-four cases of balantidiasis in Armenia during the 7 years 1927-32, of which seven were in children. Of the twenty-four patients only two had been in contact with pigs.

It may be supposed, therefore, that in addition to the domestic pig, there exist some other as yet unknown reservoirs of the pathogenic agent. POPOV and GNEZDILOV have found balantidia in wild boars, which are encountered in Armenian subtropical swamps.

Balantidial infection in rats was first encountered by NÖLLER (1926) in a rat caught in Berlin near a pigsty. This author, however, leaves open the question of species and connection with pig balantidia.

ORIGINAL OBSERVATIONS.

In studying the intestinal protozoa of Moscow rats, we found balantidia in eight rats out of a group of twenty-seven (i.e., in 29 per cent.) caught in the Elephant House of the Moscow Zoological Garden. The spontaneously infected rats were outwardly healthy. No emaciation or weakness could be noted in them and no lesions of the colon were observed upon autopsy. The intestinal walls were of normal colour but perhaps slightly anaemic. There were no ulcers or haemorrhages of any kind in the colon, the contents of which were solid. The mucous membrane of the caecum was hyperaemic and in one of the rats we found here small ulcers with uneven borders. The contents of this rat's intestine were liquid.

Under the microscope, balantidia were found in enormous numbers in the

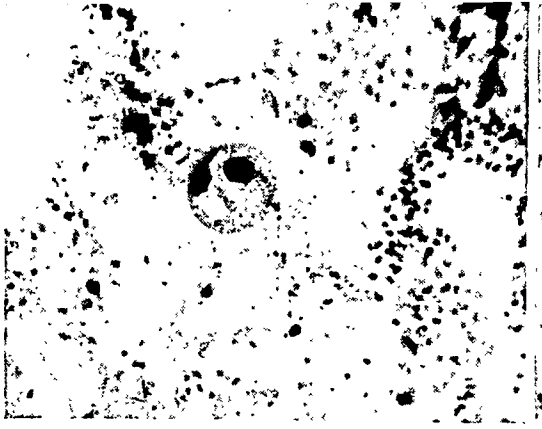


FIG. 1.

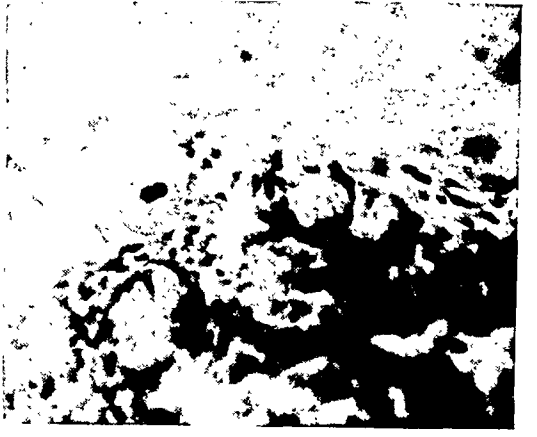
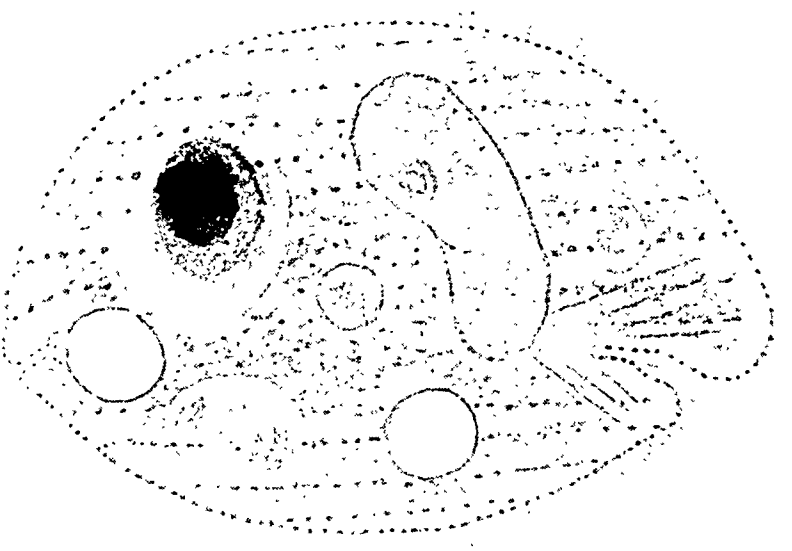


FIG. 3.

FIG. 2.



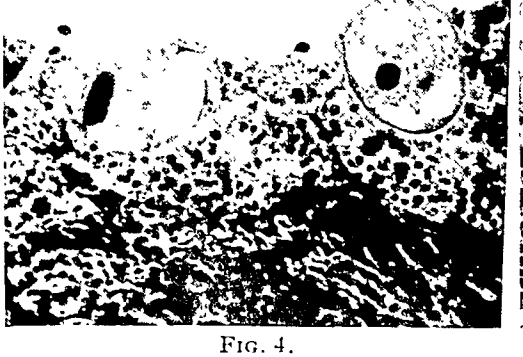


FIG. 4.

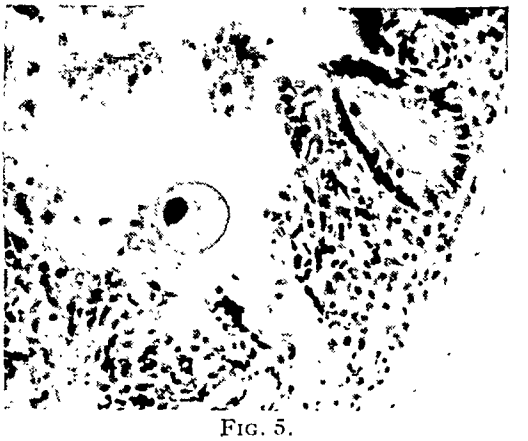


FIG. 5.

FIG. 1.—Balantidium in intestinal gland of spontaneously infected rat. (*M. decumanus*).
FIG. 2.—Balantidium from the rat.
FIG. 3.—Balantidium in submucosa of experimentally infected guineapig.
FIG. 4.—Penetration of balantidium into mucous membrane of intestine (experimental infection in rat).
FIG. 5.—Formation of ulcer in intestine of rat, experimentally infected with balantidia.

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contents of the caecum of all the eight rats. In histological sections balantidia were found in the caecal wall and in the lumina of intestinal glands. Phagocyted red cells were found only in the balantidia of the rat which showed ulcerative lesions of the caecum.

In this animal balantidia occurred in the glands, and in the lymphadenoid tissue of the submucosa, usually without any reaction in the surrounding tissues. In some places, however, balantidia were surrounded by a zone of necrotic tissue, teeming with bacteria. (Fig. 1.)

We were able to isolate cultures of balantidia in St. John's medium for amoebae and Riss's special medium for balantidia from all the eight cases. The comparative morphological study of the cultural forms of those eight strains of balantidia of rat origin, of two human and five pig strains did not show any essential differences between them.

The *Balantidium* from the rat (Fig. 2) has the same oval form as *B. coli* Malmst., a cytostome at the anterior and a cytopyge at the posterior end. As regards its structure, its cytostome and nuclear apparatus are similar to those of *B. coli*. The nucleus, usually bean-like, is more rarely sausage shaped. Sometimes its long axis is parallel to the cytopharynx, more often it is perpendicular to it. Measurements of the length and width of human, pig and rat balantidia (150 specimens of each) also did not reveal any essential differences. The average length of the *Balantidium* from the rat was about 30μ, its width about 20μ; the length-width ratio in one series was 1·06, in another 1·1.

No balantidia were found in rats caught in other parts and buildings of the Zoological Garden. With a view to elucidating the possibility of a spontaneous transmission of balantidia from pigs to rats we examined fifty rats caught in four different pigsties. No balantidia were found in any of them. On the other hand, 200 pigs examined by us at the city slaughterhouse showed a 100 per cent. infection with balantidia.

Experimental Balantidiasis.

With the object of studying in more detail the biological character of the balantidia which we had found in rats, we carried out infection experiments in white rats and guineapigs with the strains isolated from human subjects, pigs and rats.

Besides the negative results (Grassi and Calundruccio, Wising, Ziemann) with the experimental infection of men already mentioned, negative results were also obtained by Roos (1894) in dogs, by Hegner in young pigs, by Glesser in cats, by Prowazek (1913) and Hegner (1927) in rabbits and dogs.

Casagrandi succeeded in infecting with balantidia cats suffering from enteritis, while attempts to infect healthy cats gave negative results. Brumpt (1909) infected monkeys with pig balantidia and *vice versa*. Walker (1913) obtained similar results; and also infected monkeys with balantidia of human origin. Hegner (1926) infected guineapigs with balantidia from domestic

pigs, and later (1927) with cultures of these balantidia. Still later (1930) he succeeded in infecting rats by introducing the inoculum into the caecum, both with a catheter (under an anaesthetic) and directly after laparotomy. By varying the diet of the rats SCHUMAKER (1930) showed that carbohydrates rendered rats susceptible to *Balantidium* infection, while rats fed on proteins remained insusceptible to it.

OBITZ in 1931 found *Balantidium* trophozoites in eight rats out of twenty-one which had been fed with *Balantidium* cysts (pig faeces). The infected rats excreted cysts for a long time.

The balantidia of human origin utilized for the present study were obtained from a female balantidiasis patient, now living in Moscow, having come there several years ago from Leningrad. She has had much contact with pigs in the past. It is interesting to note, that our patient, like those of BADE and CALMANN, suffered also from a *Bothryocephalus latus* infection. Moreover, she was also infected with *Taenia solium*. We used also a second *Balantidium* strain of human origin, which we obtained in culture from the Central Institute of Tropical Diseases in Moscow.

Our eight rat strains were isolated from the caecal contents of the eight spontaneously infected rats, as reported above.

We utilized, moreover, five strains of pig origin; pig faeces were filtered through gauze and after this inoculated into media for cultivation, or used directly for the inoculation of the experimental animals.

The animals—rats and guineapigs—before being used in these experiments were examined on two consecutive days in order to exclude the possibility of naturally acquired balantidiasis. On the 3rd day a laxative (white bread, soaked in a saturated solution of sodium sulphate) was administered and the faeces examined for a third time. Only after all three examinations had proved to be negative were the animals used for the experiments.

For infection with cultures we took ten test-tubes of 48-hour cultures, mixed their contents and administered 5 c.c. of the mixture per anum to rats, or 10 c.c. to guineapigs. After this the anus was closed with Michel's surgical clamp for 24 hours.

To some of the animals faeces containing balantidia were administered by the mouth. The experimental animals were given the usual diet (beet, oats, bread) and were examined microscopically and by the culture method.

Out of seven attempts to infect white laboratory rats with caecal contents of spontaneously infected wild grey rats four were successful. Some of the laboratory rats thus infected began to discharge balantidia on the following day and in these cases their liquid excrements contained balantidia in large numbers. In other rats the infection had a latent character and balantidia could be discovered only in their caecal contents upon autopsy. Such latent carrying of balantidia by the rat seems to continue for a long time, since it was discovered in several cases 1 and 4 months after the inoculation.

The results are summarized in the following table :—

Balantidium from Pigs.	Animals. Total.	Number Positive.
1. Feeding of intestinal contents of the pig to white rats	4	0
2. Rectal inoculation of the same to white rats	7	1
3. Rectal inoculation of cultures to white rats	3	0
4. Feeding of cultures to guineapigs	7	0
Total	21	1
Balantidium coli (Human).		
1. Feeding of the faeces of our balantidiasis patient to white rats	3	0
2. Rectal inoculation of the same to white rats	4	0
3. Rectal inoculation of cultures to white rats	4	3
4. Rectal inoculation of cultures to guineapigs	3	1
Total	13	4
Balantidium from the Rat.		
1. Feeding of the intestinal contents of grey rats to white rats	7	0
2. Rectal inoculation of the same to white rats	6	4
3. Rectal inoculation of cultures to white rats	2	1
4. Rectal inoculation of cultures to guineapigs	4	3
Total	19	8

Out of four rectally inoculated guineapigs, three were infected. All the three discharged balantidia in large numbers. In one of them the mucous membrane of the caecum was found slightly hyperaemic upon autopsy, no ulcerative lesions were, however, discovered. Histological examination showed balantidia in the submucosa (Fig. 3).

In the tissues of both rats and guineapigs *Balantidium* does not seem to find conditions favourable to its multiplication, since the ciliates are not encountered in large groups (colonies) but as isolated individuals or in small groups of two or three ; however, not infrequently around them one finds disintegrated cells.

The penetration of balantidia into the depth of the mucous membrane, both in the spontaneously infected rats and in the experimentally infected guineapigs and rats, contradicts the assumption of DOGIEL and GNESDILOFF that the cause of such penetration is the deficiency of carbohydrate food (starch in the first place) in the host's intestine, since in all of our spontaneous and

experimental cases abundant carbohydrate food was present in the intestine, and the balantidia were packed with starch granules. This, however, did not prevent their penetration into the mucous membrane (Figs. 4 and 5).

We leave open the question as to the species of the balantidia, found by us in the rat, pending a more detailed study of the material.

Summary.

1. In examining wild rats from a single source (the Elephant House of the Moscow City Zoological Garden) for their intestinal protozoa, we found eight rats (29 per cent. of the total number) to be spontaneous carriers of balantidia, morphologically identical with human *Balantidium coli* and the pig balantidium. The examination of a large number of rats from other places and buildings of the Zoological Garden as well as of fifty rats from four Moscow pigsties revealed no infections, while in the course of an examination of 200 pigs from the Moscow City slaughterhouse these ciliates were found in every pig without exception.

2. Balantidiasis in rats has a chronic character. Several of our cases showed ulcerative lesions of the caecum and parasites in the caecal wall.

3. Attempts at experimental infection of white rats and guineapigs with various strains of *Balantidium* (human, pig and rat) seem to indicate that these animals are susceptible to all of them, being least susceptible to pig balantidia and most susceptible to those of rat origin.

4. Experimental balantidiasis in rats, like the *spontaneous* form, has mainly a chronic or latent character, the animals continuing to harbour balantidia for more than 4 months. Ulcerative lesions of the caecum, as well as the penetration of the parasites into the tissues were discovered only after infection with rat strains.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol XXXI. No 1. June, 1937.

THREE CASES OF BLACKWATER FEVER FOLLOWING THE ORAL ADMINISTRATION OF ATEBRIN.

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There is a general belief already reflected in the literature on malaria that atebrin administration is free from the risks of precipitating haemoglobinuria in potential blackwater fever patients, and to date, despite the large-scale use of atebrin, but few cases have been recorded following the exclusive administration of atebrin simplex. (MURRAY, 1934; MOIR, 1934; NÄGELSBACH, 1933.)

During the course of fluorescence analysis in mapping the distribution of atebrin in the cells of the liver, spleen and skin, we encountered three cases in which blackwater fever supervened either during or after treatment of malaria with normal standard doses of atebrin. None of the cases had taken quinine up to within 2 to 9 months before the onset of their haemoglobinuria, so that quinine can be excluded as having taken part in the precipitation of the attack of blackwater fever. (cf. MOIR, NÄGELSBACH, MURRAY, above.)

The three cases are outlined below; it is not intended to describe them fully, as their clinical and chemical findings will be reported elsewhere.

CASE HISTORIES.

Case 1.—Male, aged 5 years, seen July, 1932. Spleen 2, Hackett's notation (= enlarged to umbilicus). Parasites: *P. vivax* rings +++ (=10 or more parasites per thick film oil immersion field). No fever. Commenced oral treatment on 12th July, 1932, with 0·1 gramme of atebrin given twice a day; similar doses given on 13th and 14th July. On 15th July, 2 hours after the morning dose of atebrin, patient passed dark red urine containing haemoglobin. Blood count: R.B.C.'s = 3,850,000 per c.mm. On the night of the 15th patient was very gravely ill and jaundiced, occasional vomiting and hiccoughing. Died on the morning of the 16th. Unfortunately, it was not possible to take blood from this patient, so no information is available as to the pigments that were present in the serum. The boy last took quinine in September, 1931, presumably for malaria, and from that date until we commenced treatment with atebrin no quinine was administered.

*Dorothea Simmons Research Funds.

Case 2.—Male, aged 26 years, seen 11th January, 1935. Spleen 1, Hackett's notation (= midway between costal margin and the umbilicus). Parasites: *P. vivax* rings + (0 to 1 per thick film oil immersion field). Moderate fever (37·5° to 39° C.). Bilirubin = 0·9 mg. per cent. Blood count: R.B.C.'s = 4,490,000 per c.mm.; haemoglobin (Dare) = 14·0 grammes. Commenced oral treatment with atebrin on 12th January with 0·1 gramme three times a day for 5 days; continued until 16th January. After treatment blood negative by thick film smear. Spleen 1. Blood count: R.B.C.'s = 4,570,000 per c.mm.; haemoglobin (Dare) = 14·0 grammes; serum bilirubin = 0·8 mg. per cent. Conjunctivae and skin yellow from deposit of atebrin. On 17th January, on completion of treatment, a spleen puncture was done* and a prolonged search of both the thick and thin spleen smears failed to reveal either parasites or pigment.

On 17th January, at 10 p.m., while still in hospital, the patient passed 800 c.c. of black thick urine containing haemoglobin; blood serum contained oxyhaemoglobin and methaemoglobin†; bilirubin = 3·5 mg. per cent. Blood count: R.B.C.'s = 4,030,000 per c.mm. The skin appeared icteric so far as could be seen through deposit of atebrin. He continued to pass black or red urine during 18th, 19th and 20th January, the urine clearing gradually.

Patient was discharged on 10th February. No reappearance of parasites in blood. He had taken his last dose of quinine in October, 1934, when undergoing treatment for malaria. The case is interesting because in Salonika, during December and January, there is probably no transmission, so that the January attack of malaria was no doubt a relapse following his attack in October which had been inadequately treated with quinine.

Case 3.—Female, aged 38 years, seen on 10th March, 1935. Spleen 1. Hackett's notation. Parasites: *P. falciparum*, gametes and rings, ++++ (20 or more per thick film oil immersion field). Blood count: R.B.C.'s = 4,120,000 per c.mm.; haemoglobin (Dare) = 13·5 grammes; serum bilirubin = 1·2 mg. per cent. Patient was running temperatures of 38·5° C. to 40° C. Commenced oral treatment with atebrin on 11th March with 0·1 gramme three times a day for 5 days until 15th March. At the end of treatment blood was negative. Blood count: R.B.C.'s = 4,110,000 per c.mm.; bilirubin—0·8 mg. per cent. No yellowing of skin or conjunctivae. Temperature now normal. On 16th March a spleen puncture was done and showed no parasites or pigment. On 17th March patient was discharged from hospital at midday, but returned the following morning, having passed red urine during the night of the 17th-18th. Blood count on 18th March: R.B.C.'s = 3,840,000 per c.mm.; serum bilirubin = 1·7 mg. per cent. Serum contained oxyhaemoglobin only. She passed one other specimen of red urine on 18th March in the evening, after which the urine cleared.

P. falciparum reappeared in this patient's blood on 23rd March, and she was treated with 1 gramme injections of quinine bihydrochloride intramuscularly for 3 days, followed by 0·25 gramme injections for three days. She was discharged on 4th April.

This patient had taken her last dose of quinine before the onset of the blackwater fever on 17th March, sometime in October, 1934, and she probably represented one of the early spring relapses, common in Greece.

COMMENT.

These results, together with other spleen punctures in blackwater fever that will be reported elsewhere, tend to throw some doubt on the view that the onset of blackwater fever is associated with the sudden destruction of large

*We were carrying out at this time a series of spleen punctures in cases of blackwater fever and malaria with the object of correlating the results of spleen puncture and peripheral blood examination; the results of this work are now in the press.

†In the light of more recent research it would seem that this pigment was not methaemoglobin, but the new pigment of FAIRLEY & BROMFIELD (1934) which has been named by them pseudo-methaemoglobin (1937).

numbers of parasites (MANSON-BAHR, 1924; BLACKLOCK & MACDONALD, 1928) and to support the view expressed by YORKE, MURGATROYD & OWEN (1930). Further, it is our opinion based on a large series of spleen punctures in malaria and blackwater fever that in both *vivax* and *falciparum* infections an examination of spleen puncture material is rarely a better index of malaria infection than is examination of peripheral blood by thick films (cf. WENYON, 1926; THOMSON, 1924). Our results do not support the view that in *falciparum* infections peripheral blood examination is not a good index of infection. It is not intended to suggest that in the cases above there was no infection with malaria, since in Case 3, the subsequent appearance of *falciparum* proved that this was not so (re-infection could be ruled out in this case owing to the time of the year and the location of the patient), but we do claim that the infection was an extremely light one, a view amply confirmed by the spleen findings.

In both these cases the spleen puncture was carried out some 12 to 24 hours before the attack of blackwater, and although plenty of splenic material was present, a prolonged search of both the thick and thin spleen smears failed to reveal either parasites or pigment.

It would appear from these cases that, like quinine and plasmoquine, atebrin is capable of firing off an attack of blackwater fever. In a previous paper FOY and KONDI (1936) stated that in their opinion atebrin was not contra-indicated in cases of blackwater fever where parasites were present and needed attention and, despite the present report, we still incline to this view. It is frequently stated that it is wise to withhold quinine in cases of blackwater fever, but here in Greece the " treatment " in a great many cases includes massive oral, intramuscular or intravenous doses of quinine (0·2 to 0·5 gramme) and the results, judging from the death rates, compare favourably with any other so-called " treatment." Our feeling is that if parasites are present and are giving rise to clinical symptoms, then they should be dealt with. For this purpose either quinine or atebrin may be used provided, of course, it has been previously established that the patient is not sensitive to either of these drugs.

As will be shown in a later paper, the vast majority of patients with haemoglobinurias in Greece have taken either a large or a small amount of quinine sometime within 3 days prior to their first passage of black urine, yet the great majority of these cases are " treated " during their attack with large doses of quinine with apparently no ill-effects. It would seem then that although quinine can be proved to have been taken immediately before the onset of the blackwater, and generally regarded as having fired off the attack, this does not mean that quinine need be withheld during treatment if parasites are present and causing trouble. We have seen many heavily infected patients with high fever whose blackwater attacks were reputed to have been precipitated by quinine, yet who were subsequently treated successfully by massive doses of quinine.

Case 2.—Male, aged 26 years, seen 11th January, 1935. Spleen 1, Hackett's notation (= midway between costal margin and the umbilicus). Parasites: *P. vivax* rings + (0 to 1 per thick film oil immersion field). Moderate fever (37·5° to 39° C.). Bilirubin = 0·9 mg. per cent. Blood count: R.B.C.'s = 4,490,000 per c.mm.; haemoglobin (Dare) = 14·0 grammes. Commenced oral treatment with atebrin on 12th January with 0·1 gramme three times a day for 5 days; continued until 16th January. After treatment blood negative by thick film smear. Spleen 1. Blood count: R.B.C.'s = 4,570,000 per c.mm.; haemoglobin (Dare) = 14·0 grammes; serum bilirubin = 0·8 mg. per cent. Conjunctivae and skin yellow from deposit of atebrin. On 17th January, on completion of treatment, a spleen puncture was done* and a prolonged search of both the thick and thin spleen smears failed to reveal either parasites or pigment.

On 17th January, at 10 p.m., while still in hospital, the patient passed 800 c.c. of black thick urine containing haemoglobin; blood serum contained oxyhaemoglobin and methaemoglobin†; bilirubin = 3·5 mg. per cent. Blood count: R.B.C.'s = 4,030,000 per c.mm. The skin appeared icteric so far as could be seen through deposit of atebrin. He continued to pass black or red urine during 18th, 19th and 20th January, the urine clearing gradually.

Patient was discharged on 10th February. No reappearance of parasites in blood. He had taken his last dose of quinine in October, 1934, when undergoing treatment for malaria. The case is interesting because in Salonika, during December and January, there is probably no transmission, so that the January attack of malaria was no doubt a relapse following his attack in October which had been inadequately treated with quinine.

Case 3.—Female, aged 38 years, seen on 10th March, 1935. Spleen 1. Hackett's notation. Parasites: *P. falciparum*, gametes and rings, ++++ (20 or more per thick film oil immersion field). Blood count: R.B.C.'s = 4,120,000 per c.mm.; haemoglobin (Dare) = 13·5 grammes; serum bilirubin = 1·2 mg. per cent. Patient was running temperatures of 38·5° C. to 40° C. Commenced oral treatment with atebrin on 11th March with 0·1 gramme three times a day for 5 days until 15th March. At the end of treatment blood was negative. Blood count: R.B.C.'s = 4,110,000 per c.mm.; bilirubin—0·8 mg. per cent. No yellowing of skin or conjunctivae. Temperature now normal. On 16th March a spleen puncture was done and showed no parasites or pigment. On 17th March patient was discharged from hospital at midday, but returned the following morning, having passed red urine during the night of the 17th-18th. Blood count on 18th March: R.B.C.'s = 3,840,000 per c.mm.; serum bilirubin = 1·7 mg. per cent. Serum contained oxyhaemoglobin only. She passed one other specimen of red urine on 18th March in the evening, after which the urine cleared.

P. falciparum reappeared in this patient's blood on 23rd March, and she was treated with 1 gramme injections of quinine bihydrochloride intramuscularly for 3 days, followed by 0·25 gramme injections for three days. She was discharged on 4th April.

This patient had taken her last dose of quinine before the onset of the blackwater fever on 17th March, sometime in October, 1934, and she probably represented one of the early spring relapses, common in Greece.

COMMENT.

These results, together with other spleen punctures in blackwater fever that will be reported elsewhere, tend to throw some doubt on the view that the onset of blackwater fever is associated with the sudden destruction of large

*We were carrying out at this time a series of spleen punctures in cases of blackwater fever and malaria with the object of correlating the results of spleen puncture and peripheral blood examination; the results of this work are now in the press.

†In the light of more recent research it would seem that this pigment was not methaemoglobin, but the new pigment of FAIRLEY & BROMFIELD (1934) which has been named by them pseudo-methaemoglobin (1937).

numbers of parasites (MANSON-BAHR, 1924; BLACKLOCK & MACDONALD, 1928) and to support the view expressed by YORKE, MURGATROYD & OWEN (1930). Further, it is our opinion based on a large series of spleen punctures in malaria and blackwater fever that in both *vivax* and *falciparum* infections an examination of spleen puncture material is rarely a better index of malaria infection than is examination of peripheral blood by thick films (cf. WENYON, 1926; THOMSON, 1924). Our results do not support the view that in *falciparum* infections peripheral blood examination is not a good index of infection. It is not intended to suggest that in the cases above there was no infection with malaria, since in Case 3, the subsequent appearance of *falciparum* proved that this was not so (re-infection could be ruled out in this case owing to the time of the year and the location of the patient), but we do claim that the infection was an extremely light one, a view amply confirmed by the spleen findings.

In both these cases the spleen puncture was carried out some 12 to 24 hours before the attack of blackwater, and although plenty of splenic material was present, a prolonged search of both the thick and thin spleen smears failed to reveal either parasites or pigment.

It would appear from these cases that, like quinine and plasmoquine, atebrin is capable of firing off an attack of blackwater fever. In a previous paper FOY and KONDI (1936) stated that in their opinion atebrin was not contra-indicated in cases of blackwater fever where parasites were present and needed attention and, despite the present report, we still incline to this view. It is frequently stated that it is wise to withhold quinine in cases of blackwater fever, but here in Greece the " treatment " in a great many cases includes massive oral, intramuscular or intravenous doses of quinine (0·2 to 0·5 gramme) and the results, judging from the death rates, compare favourably with any other so-called " treatment." Our feeling is that if parasites are present and are giving rise to clinical symptoms, then they should be dealt with. For this purpose either quinine or atebrin may be used provided, of course, it has been previously established that the patient is not sensitive to either of these drugs.

As will be shown in a later paper, the vast majority of patients with haemoglobinurias in Greece have taken either a large or a small amount of quinine sometime within 3 days prior to their first passage of black urine, yet the great majority of these cases are " treated " during their attack with large doses of quinine with apparently no ill-effects. It would seem then that although quinine can be proved to have been taken immediately before the onset of the blackwater, and generally regarded as having fired off the attack, this does not mean that quinine need be withheld during treatment if parasites are present and causing trouble. We have seen many heavily infected patients with high fever whose blackwater attacks were reputed to have been precipitated by quinine, yet who were subsequently treated successfully by massive doses of quinine.

These case records indicate that haemoglobinuria can follow the administration of atebrin given for malaria, just as it may that of quinine or plasmoquine, but it should not be inferred from this that these specific drugs are the direct cause of the haemoglobinuria, or that they are contra-indicated in haemoglobinuric cases provided parasites are found and the patient has no idiosyncrasy to the drugs.

Summary and Conclusions.

1. Three cases of blackwater fever following the oral administration of atebrin simplex are recorded in patients who had taken no quinine for months previous to their attack of haemoglobinuria.

2. In two instances the onset occurred on the 1st and 3rd days after a full course of atebrin (i.e., 1·5 grammes spread over 5 days). In the other case haemoglobinuria appeared in a boy of 5 years on the 4th day of treatment, after he had received 0·7 gramme.

3. It follows that atebrin, like quinine and plasmoquine, is capable of precipitating blackwater fever in certain patients, but whether these drugs are all equally potent in this respect, remains to be determined.

4. In Cases 2 and 3 the haemoglobinuria occurred on a blood devoid of parasites, as shown by thick film of peripheral blood and spleen puncture.

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TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

YELLOW FEVER IMMUNE BODIES IN THE BLOOD OF AFRICAN PRIMATES.

BY

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AND

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In a preliminary communication (FINDLAY *et al.*, 1936) it was reported that, as shown by the mouse protection test, yellow fever immune bodies had been detected in the blood serum of three out of twenty-five African primates. The positive sera were obtained from a chimpanzee, *Anthropopithecus troglodytes* L., from French Guinea, a baboon, *Papio* sp., from the Belgian Congo and a colobus monkey from Goaso, Gold Coast. This colobus has since been found to belong to a new species *Procolobus badius waldroni* HAYMAN, 1936.

*Our grateful thanks for assistance in obtaining monkey bloods are due to Dr. E. D. PRIDIE, Dr. A. E. HORGAN, Dr. R. KIRK, and Dr. N. CORKILL, Anglo-Egyptian Sudan; Dr. W. H. KAUNTZE and Dr. D. S. DE BOER, Uganda; Mr. R. DAUBNEY, Kenya; Dr. D. DUFF, Dr. A. MACPHERSON and Mr. G. S. CAUSDALE, Gold Coast; Mr. MOREY; and Dr. P. D. OAKLEY and Mr. D. H. S. DAVIS, Sierra Leone. We desire to thank Dr. S. ZUCKERMAN and Captain GUY DOLLMAN for their help in regard to the nomenclature of the primates.

One of us, F.O.M., is in receipt of a grant from the Medical Research Council.

The sera of Asiatic monkeys, rhesus (*Macaca mulatta*) and crab-eating macaques (*Macaca irus*) were negative. In South America SOPER (1936) also found that of the sera of ninety-three monkeys, captured in yellow fever areas, eighteen or approximately 20 per cent. gave a positive reaction with the mouse protection test for immunity to yellow fever. The monkeys with positive sera were three *Callicebus ornatus* and one squirrel monkey, *Saimiri sciureus* L. from Restrepo, Colombia, and fourteen capuchin monkeys (*Cebus apella* ?) from Matto Grosso and Minas Geraes.

YELLOW FEVER IMMUNE BODIES IN THE SERA OF AFRICAN MONKEYS.

Locality.	Species.	Number of Sera Examined.	Number of Sera Protecting.	Number of Sera not Protecting.
Anglo-Egyptian Sudan	*Galago senegalensis senegalensis* Elliot	3	0	3
	Erythrocebus patas Elliot	1	0	1
	Cercopithecus aethiops centralis Neumann	15	1	14
Sierra Leone	*Colobus vellerosus* Elliot (= *polykomos* Schwarz)	1	1	0
Liberia	*Cercopithecus* sp.	10	4	6
Gold Coast	*Cercopithecus* sp.	1	0	1
	C. diana diana	1	1	0
	Erythrocebus patas Elliot	1	0	1
	Colobus vellerosus Elliot (= *polykomos* Schwarz)	5	3	2
	Procolobus badius badius Pocock, 1935	6	3	3
	P. badius waldroni Hayman	3	1	2
Uganda	*Cercopithecus aethiops centralis* Neumann	20	5	15
	Total	67	19	48

In the present communication further observations are recorded in regard to the presence of yellow fever immune bodies in the blood of wild African monkeys. All the sera were tested by the intraperitoneal mouse test in a dilution of 1 in 8. Sera which gave a positive reaction at the first test were retested at least once, more often twice. The results are shown in the table. Of sixty-five monkeys, seventeen or approximately 25 per cent. were found to have immune bodies in their blood. As controls to those monkeys, the bloods of six monkeys (*Cercopithecus aethiops johnstoni*) were examined from an area in Kenya where immune bodies have never been detected in the bloods of human beings. All

these Kenya monkeys gave negative results. Ten rhesus monkeys from India also gave negative reactions when their sera were examined by the mouse protection test. These negative results, combined with those previously recorded, suggest that, in so far as the blood of primates is concerned, the mouse protection test is probably as specific for yellow fever immune bodies as it is in the case of human bloods.

The monkeys with positive sera from the Gold Coast all come from the forest region of Ashanti, more especially the country round Juaso ; those from Uganda were obtained from the northern part of the country, from Kisunga, Kibanda : Kisuna, Katulikire : Kirwala : and Kiroko, Mumyoka ; the Sudanese monkeys in all probability, came from Sennar, with the exception of the lemurs (*Galago senegalensis senegalensis*) which were obtained in the Nuba mountains.

It is of interest to note that yellow fever immune bodies have been detected in the bloods of human beings from all the areas in Africa where monkey bloods are also positive, with the exception of the Blue Nile Province of the Anglo-Egyptian Sudan : human sera from this locality are now under investigation.

If the results recorded in the present communication are combined with those previously reported it will be seen that twenty-two positive sera were obtained from ninety-two primates, or approximately 23 per cent., a figure which agrees closely with that obtained in South America.

If the mouse protection test is specific for yellow fever immune bodies and, so far as the blood of primates is concerned, there is no evidence to contradict its specificity—the results suggest that a fifth of the wild monkeys from areas in the Old and New Worlds where yellow fever is endemic have at some time or another been infected with the yellow fever virus, although this infection is not as a rule fatal. In Africa and South America the monkey populations thus infected must run into many millions : failure of the susceptible human host, either through lack of numbers, or through the development of immunity, may not therefore necessarily eliminate infection from an area. On the other hand, even if monkeys act as an alternative host for the yellow fever virus, the problem of how the disease maintains itself in an endemic area is by no means solved, for the virus is present in the peripheral blood stream and thus in a condition to be transferred to mosquitoes, for at the most, 4 or 5 days ; the life of an individual mosquito is not long and monkeys do not appear to roam over large tracts of country. Unfortunately, little or nothing is known in regard to the life span of aedine mosquitoes under natural conditions, while further work is obviously required on the œcology of monkeys, more especially in regard to their ectoparasites and to the extent of territory over which they roam.

An additional point which requires investigation is the extent to which *Aëdes aegypti* feeds on wild monkeys or other animals as an alternative to human beings. Certain monkeys are known to live in the vicinity of houses and to raid cultivated fields, but it is open to question whether this association with man

is sufficiently close to allow a fifth of African monkeys to be bitten by mosquitoes as domesticated as *A. aegypti*: other mosquitoes must be regarded as possible potential vectors of yellow fever under natural conditions.

Conclusions.

1. An examination by the mouse protection test of the sera of sixty-seven wild African monkeys, from areas where immune bodies have been found in the blood of human beings, showed the presence of yellow fever immune bodies in nineteen.

2. Of a total of ninety-two African monkeys so far investigated, twenty-two or approximately 23 per cent. have shown yellow fever immune bodies.

3. The Old World monkeys with positive sera include at least seven different species: *viz.*, the chimpanzee *Anthropopithecus troglodytes*; baboon *Papio* sp.; *Cercopithecus aethiops centralis*; *C. diana diana*, *Colobus vellerosus* (= *polykomos*), *Procolobus badius badius* and *P. badius waldroni*.

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TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

LEPROSY IN THE SOUTHERN SUDAN.

BY

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The measures taken for the control of leprosy in the Southern Sudan are of peculiar interest. The disease appears to be a serious problem in the region between the 6th and 12th parallels and west of the Nile, especially in the watershed dividing the sources of the Congo from those of the Nile. This is described by O. F. H. ATKEY (1934) as a region infested by *Glossina morsitans.*

He inclines towards ascribing the greater incidence of leprosy in this area to two factors: the scarcity of milk, and the closer contact and mixing of the people with outsiders as compared with the nomadic tribes among whom leprosy is rare, and who have abundant milk and very little intercourse with others. This is interesting as precisely the same two factors have been given as accounting for the low rate of leprosy in the plains of the Punjab, as compared with other parts of India.

COCHRANE (1931) mentions that the disease may be spreading from the Belgian Congo, and this would appear to be not unlikely considering the very high incidence in that country.

H. M. WOODMAN (1937) in an article in these TRANSACTIONS has described the result of treatment in the largest of three leper settlements in this area with a population of 2,000 patients. This took the form of mass treatment with various soluble preparations of chaulmoogra oil, more selective treatment being found impossible through lack of staff. Treatment also included painting macular and nodular areas with trichloracetic acid.

The results are put forth in four tables, the first two comparing treated and untreated cases after 3 years, and the other two comparing treated and untreated cases after a 6 years period. Unfortunately, the terms used in describing progress are misleading. The writer uses the words "quiescent" and "arrested," but apparently in quite a different sense from that agreed upon at the Leonard Wood Memorial Conference in 1931, and now generally adopted. The word "cured" is also applied to cases "in which no visible lesion is any longer apparent," a most fallacious criterion of *improvement*, let alone *cure*. In the 6-year period 2·8 per cent. of untreated cases are "cured," and 46·4 per cent. "arrested." In the same period, out of the treated cases 0·7 per cent. are "cured" and 10·5 per cent. are "arrested." On the other hand, the untreated include under "quiescent" and "improved" an aggregate of 41·3, compared with 58·7 among the treated.

From this the conclusion that "generally speaking the results weigh clearly in favour of treatment" is scarcely justified. It is perhaps somewhat doubtful to what extent special treatment has been beneficial as compared with the benefit derived from the care and protection provided by settlement life.

Leprosy is one of the most difficult diseases to treat. It is becoming generally recognised that the main treatment consists, as in tuberculosis, in improving the general health of the patient. When that has been satisfactorily accomplished carefully applied special remedies form a very useful additional aid towards recovery.

In classifying causes and in judging of their progress, bacteriological examination, carefully and repeatedly carried out, is necessary if results of any value are to be obtained. Appearances, though useful, are apt to be fallacious if not checked up by the microscope.

The classification and terminology at present accepted with regard to leprosy is no doubt open to criticism ; but to avoid confusion it would be well to adhere to that in current use till changed or modified by general consent.

Apart altogether from treatment, the measures taken to control leprosy in the Southern Sudan are in many ways unique. The population is so sparse and scattered that methods applicable in more densely populated areas would have been quite unsuitable in this area. A survey (CRUICKSHANK, 1932) was first made in the Yambio District, which showed 1,556 cases in a population of 58,136, or 2·8 per cent. A suitable area for a settlement was selected and 100 cases at first admitted, and thereafter " the trickle of admissions slowly grew into a stream and then into a flood. At one time fifty a day were being admitted, and as each case takes some 15 minutes to register, the task entailed can be imagined, and the hut-builders and road-makers were strained to the utmost to keep pace with the demand. Within 18 months of starting, 2,700 cases were admitted, housed and under treatment ; by then, resources and staff could cope with no more, and it was felt wiser to cease admission and consolidate the position."

The *Report of the Sudan Medical Service for 1935*, states that there were 2,698 lepers in camps and settlements, and 1,512 were under observation and treatment. " The stage has been reached where further treatment with chaulmoogra derivatives is of no avail, in so far as this applies to cases prior to 1933-34. It is particularly worthy of note that, whereas in 1932 31 per cent. of lepers had been rendered quiescent, by 1935 the percentage is 78. A big proportion of early cases becomes arrested in any case, but is assisted to do so by treatment. No form of treatment yet tried holds out much prospect for the advanced cutaneous and mixed cases. They have all undergone prolonged treatment, and the very few who have improved would have probably done so without interference. The most hopeful outlook for the majority of C3 cases who survive is the gloomy prospect of the maimed and disabled N2 stage. In spite of the great incidence of leprosy, it is important to note (1) that less than 10 per cent. of cases are any danger to their neighbours as far as can be at present judged, in the absence of exhaustive and repeated laboratory examinations, (2) that treatment is effective and worth trying in a large percentage of active early cases over a period not exceeding 4 years, (3) that the settlements have removed the chief foci of infection in the district, and both centralized and simplified their control."

Thus the prospect of control in this area seems bright provided that vigilance over existing cases is continued and that a careful guard is maintained against invasion from highly leprous areas in the neighbourhood such as the Belgian Congo.

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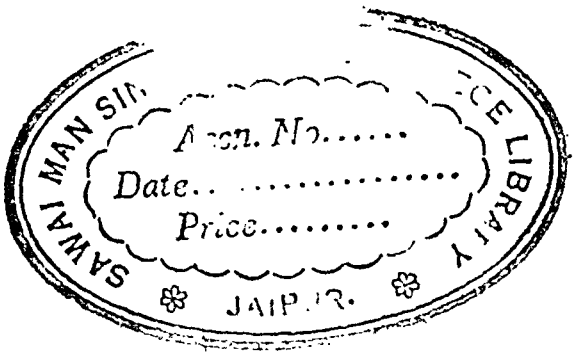
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TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.



A PRESUMPTIVE CULICINE HOST OF THE HUMAN MALARIA PARASITES.

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The conception that host efficiency is dependent upon environmentally determined biochemical factors—as well as upon the influences (such as the racial habit of feeding on blood) which determine genetic constitution (WILLIAMSON, 1928)—is as fundamental for parasitological theory as quantum mechanics is for the theory of radiation. Lacking either, phenomena can be explained only up to the point—if yet fundamentally at all, in parasitology—(HUFF, 1934) reached by classical doctrine. In order to test the question in

*We wish to express our obligation to successive Senior Health Officers, Penang, for opportunity to carry out our work, and further especially to Dr. J. W. SCHARFF for his critical interest in it.

relation to malaria, during the past 17 months we have afforded opportunity of infection to *Culex bitaeniorhynchus*, which is unique in that its larvae are always found among *Spirogyra* (SENIOR-WHITE, 1926) upon which they feed. Its potentialities as a malaria carrier, together with those of most other culicine mosquitoes, have been disregarded because of the belief that the parasites can complete their cycle of development only in members of the genus *Anopheles*. The species was, however, preindicated as a possible carrier by one of us some years ago (WILLIAMSON, 1927) because it breeds in pure water. As then pointed out, and since confirmed from COVELL'S valuable tables (COVELL, 1927 and 1931), many of the most notorious anopheline vectors, some of which are common associates of *C. bitaeniorhynchus*, likewise do so, thereby avoiding larvally derived taints likely to inhibit the development of the malaria parasites.

Thirty laboratory-bred females were allowed to feed once upon donors whose blood was exceptionally rich in infective gametocytes (2·3 to 10·3 per 100 leucocytes). These mosquitoes were, therefore, provided with an unusually good opportunity of infection, and this fact should be borne in mind in interpreting our findings.

Our observations, a preliminary note upon which has appeared in *Nature* (WILLIAMSON and ZAIN, 1937), have been made in the course of related daily work which has permitted comparison with both naturally and artificially infected anophelines. The results are briefly summarized below. Forms indistinguishable by us from, and indicative of, the completed insect cycle of development of the malaria parasites, were recorded in the following categories of infection, namely, sub- and benign-tertian separately and mixed; and quartan (providing over eight gametocytes per one hundred leucocytes) combined with sevenfold less intense sub-tertian. Sub-tertian infection largely predominated and the figures relating to it are given in brackets: thirty (twenty-three) insects were used; twenty-nine (twenty) plasmodial cysts were found in fourteen (eleven), the greatest number in one being seven (with others degenerated and doubtful); eighteen (fourteen) of these cysts were pigmented, the remainder being diagnosed by type, and by their containing, or being in proximity to, presumptive sporozoites; eight (six) of the fourteen (eleven) susceptible insects having cysts were infected, most of them lightly, with sporozoites in their salivary glands, two of these (one sub-tertian and one quartan sub-tertian) however revealed numerous sporozoites elsewhere. ROSS'S black spores were found in five (four) cases, one being a benign-tertian infection; sporozoites in the glands and one or more cysts were present in each of the five. Since these features are held to be individually diagnostic, the fact of their associated recurrence several times over must be accorded added evidential value. The picture presented by thirty-one unblooded laboratory-bred females used as controls presented a striking contrast, except in one respect. In several cases bodies resembling sporozoites were observed on the hind guts (but none in or near cysts). These appearances must probably

be credited to the unrecorded generalized protozoal infestations of tropical mosquitoes, which urgently call for authoritative investigation. One of us (M. Z.) found, and we have jointly confirmed, heavily brown and black pigmented cysts in unblooded laboratory-bred male anopheles (once in close association with sporozoite-like bodies present on the mid gut surrounding the cysts), a fact which annuls one or other of the current preconceptions, that (with the exception of the allied *Haemoproteus* of the pigeon, etc.) in the insect cycle allow pigment only to the genus *Plasmodium*, and to the latter a cycle confined to adult mosquitoes, disregarding the possibility of larval phases derived (cannibalistically or otherwise) from infected females dead upon the water after oviposition. These intrusive facts and possibilities must be balanced against the evidence of the differential record in the infected and uninfected series of *C. bitaeniorhynchus*. With the exception of five brown pigment granules distributed between two cysts in separate insects, no pigmented cysts, nor any black spores, were seen in our controls. These contrasts and the above indicated correlations in the infected series appear to leave little doubt that the developmental cycle of the two common malaria parasites and probably that of the quartan parasite also, can proceed to completion in this culicine. But in the absence of proof of the transmission of malaria through it, demanded by KOCH'S final postulate, no assured epidemiological conclusion is at present warranted.

The search for vectors needs to be guided by inductive principles and biochemical reasoning. Theoretically considered, if freedom from contamination of larval food and environment is favourable to the development of the malaria parasites in mosquitoes, it is not impossible that other bloodsucking insects, such as *Simulium* which breed along with them in pure water, may be carriers. This is the less unlikely since the host of the allied *Haemoproteus* of the pigeon is not a mosquito but a hippoboscid fly. The fact that the latter, together with the otherwise unrelated ticks, tsetse flies, etc., are nourished solely by blood throughout their life cycle, appears to determine their hereditary aptitude for serving as the hosts of protozoal blood parasites (WILLIAMSON, 1928), probably because the amino-acid (ROSEDALE, 1928), etc., composition of their tissues has come to resemble that of the blood which has been their sole racial food through a prolonged evolutionary epoch. Insects like mosquitoes, which feed only intermittently upon blood, and have a free and promiscuously feeding larval stage, seem to require that it is passed in well oxygenated surroundings (WILLIAMSON, 1928 and HUFF, 1934) which provide pure and vital food, if they are to be effective hosts of the human malaria parasites. In both their human and insect phases these seem to demand freedom from the products of bacterial contamination accompanied by a high level of oxidation (SENIOR-WHITE, 1926) in the nitrogen cycle (WILLIAMSON, 1935) a circumstance which suggests that in virtue of its reducing power, ascorbic acid (ARMENTANO, 1936) may possess parasiticidal or inhibitive properties. Whatever may be their solution, the

fundamental problems of malaria are thus seen to be conditioned by environmentally or genetically (HUFF, 1931) determined biochemical factors. While they are shared by both of us, the senior author is alone responsible for these opinions.

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TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

NOTES ON THE VIABILITY OF *ANOPHELES COSTALIS* OVA SUBJECTED TO NATURAL DESICCATION.

BY

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INTRODUCTION.

Eggs of certain species of culicines are more or less resistant to drought and adverse conditions of temperature, and may remain out of water for several days without their vitality being in any way impaired.

In the case of *Aëdes aegypti*, eggs have been known to hatch out after they had stood ordinary temperature, out of water, for several months.

It was, therefore, thought interesting to ascertain how the ova of our anophelines would stand desiccation. With this end in view, eggs of *Anopheles costalis* were experimented with. They were chosen merely because they were less difficult to obtain than those of the other species.

EXPERIMENTS.

The eggs used in the first part of these experiments (Table I) were collected on the coast at Drain-Marie, near Roche-Bois, in brackish water. As each egg was lifted off the surface of the drain water, it was floated on water in a specimen tube and, when the required number had been obtained, the water in the tube was siphoned off and the eggs allowed to remain stranded against the sides. The tube was then placed standing on the hot sand, at the side of the drain, in bright sunlight. After 15 minutes, all traces of water had evaporated. From that moment onwards, the tube was left uncorked. Several tubes were prepared in this way. Some were kept in the laboratory during the day, when drying in shade was required; others were left outside in the sun, when the effect of sunshine

TABLE I.

Experiment Number.	Number of Ova Used.	Whether dried Immediately or Not.	Drying Period in Hours.	Date of Floatation.	Date of Hatching and Number.	Percentage Hatched Out.
1	10	Kept on water for 24 hours	48 in shade	5/5	6/5 one 8/5 two 9/5 three	60
2	20	Dried immediately	24 in sun	6/10	8/10 four 9/10 five	45

was being tested. After the required period of exposure had elapsed, the eggs were carefully detached from the sides of the tubes and were floated on water in bowls, where they were allowed to remain until hatching took place, or until the end of the experiments.

When I found that after 2 days of drying the eggs still hatched the importance of the fact, from the point of view of anopheline control, presented itself, and I decided to alter the *modus operandi* and to start another series of experiments by imitating as far as possible what takes place in nature with regard to garden ponds, ornamental basins and other large containers which usually breed *A. costalis.*

As I could no longer obtain eggs at Drain-Marie, I had recourse to another breeding ground at Vallée-des-Prêtres. This time, it was a fresh-water basin in which spirogyra grew abundantly.

Instead of allowing the eggs to dry in a tube, as before, they were immediately brought to the experimental station and were floated on water in small concrete basins. The water was then immediately siphoned off and the eggs allowed to dry for various periods as before, after which water was poured into the basins. These had been placed outside, in such a manner that they were in the sunshine for the greater part of the day—about 5 hours (Table II).

For the benefit of those who are unfamiliar with anopheline eggs and their behaviour under certain conditions, some explanation is at this stage necessary. When an anopheline lays its eggs directly on water in a basin, or other container, these are deposited at the margin of the water. Surface tension comes into play, and the eggs are immediately attracted to the sides of the vessel. If, as is rarely the case, the ova are deposited at a short distance from the sides, even weak wind draughts soon drive them to the water's edge where they find themselves in the position mentioned above. Now, when a basin is emptied, the eggs, instead of going right down with the water as its level lowers, remain behind, stranded on the sides of the basin and when this is refilled, the eggs float again. If the time elapsed between emptying and refilling is short, all the eggs come to the surface. After 2 to 3 days of exposure to the sun, roughly 33 per cent. continue to adhere to the sides of the vessel, after water has been poured in. Of that number, some come to the surface again after several hours when, finally, about 25 per cent. remain under water.

Now to return to our drying experiments, let us examine the following Tables. In Table I are the results arrived at with the eggs kept in tubes, and Table II shows the figures obtained for the ova experimented with in the second manner.

In these tables, 48 hours in the sun for instance, does not mean 48 hours of exposure to sunlight. What is meant is that during a 48 hour period the eggs were in the sun during the day.

The tables show us that *A. costalis* ova continue to hatch out after a drying period of 72 hours. This is an important fact to bear in mind, when dealing with certain anopheline breeding grounds. In the past, whenever anopheline larvae were encountered in garden reservoirs, basins, tanks, watering pools and the like, these were emptied and allowed to dry during the day, after which they were refilled and almost invariably forgotten. The result was that even if

larvae were killed ova continued to hatch out unnoticed and gave rise eventually to adult mosquitoes.

In the above experiments, the larvae which hatched out were bred. Most of them continued to develop normally, the final adults being full size. The percentage of deaths which occurred was not higher than is usual in any breeding experiment. In order to avoid making the tables unnecessarily complicated the details of the breeding have not been given.

Wherever anopheline larvae are found, one must not forget that eggs are in most cases present and that, if drying is the only means that can be resorted

TABLE II.

Experiment Number.	Number of Ova Used.	Whether dried Immediately or Not.	Drying Period in Hours.	Date of Floatation.	Date of Hatching and Number.	Percentage Hatched Out.
1	60	Kept on water for 24 hours	48 in shade	13/10 Water poured in basin; 33 eggs come to surface immediately	15/10 ten 16/10 five 18 eggs remain under water	25
2	40	Dried immediately	24 in sun	18/10 Water poured in; 20 eggs rise immediately	19/10 six 20/10 ten 13 eggs remain under water	40
3	20	Kept on water for 24 hours	48 in sun	24/10 Water poured in	25/11 two 26/11 three	25
4	50	Kept on water for 24 hours	72 in sun	25/10 Water poured in	27/11 two 28/11 two	8
5	24	Dried immediately	96 in shade	19/2 Water poured in	None hatched	
6	21	"	"	2/2 Water poured in	"	

to for dealing with the breeding ground, a 4-day period is essential; failing this, a second drying, after a week's interval, becomes necessary. This is an important aspect of the fight against anophelines, for in Mauritius the great majority of *A. costalis* breeding places are man-made and consist almost entirely of garden pools, the water from which is used for watering vegetables so that kerosene cannot be employed.

CONCLUSIONS.

Eggs of *Anopheles costalis* allowed to dry naturally, may continue to hatch out after a drying period of 72 hours, but cease to do so after 4 days' drying.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 1. June, 1937.

A METHOD OF LABELLING AND PROTECTING BLOOD FILMS.

BY

P. TATE, PH.D.

From the Molteno Institute, University of Cambridge.

In view of the well known difficulty of labelling blood films so that the labels are not injured by immersion oil or xylol, it may be of interest to describe a method which has been found to be very satisfactory.

The labels, ungummed, are fixed to the slide by means of cellulose lacquer or other similar cellulose preparation. The description of the slide is written with Indian ink in the usual manner, and, when dry, the whole label is covered with a thick coating of the cellulose preparation. When properly done, such labels are uninjured by either immersion oil or xylol and they are very firmly affixed to the slide.

It has been found that the blood films themselves may be protected with a similar coating of the cellulose without apparently interfering with the microscopic definition of the blood cells or organisms therein. Such protected slides have proved very valuable for class work as they are well protected from dust and scratching and no special precautions need to be taken in washing off the immersion oil after use.

The method has been tested on films stained both by Giemsa's and Leishman's methods. The films have been kept for more than a year after treatment with the cellulose and the stain has not faded more than in similar films kept uncoated.

Several different commercial cellulose preparations have been tested, but that which has given the best results is the preparation sold in collapsible tubes under the trade name " Durofix." The simplest way to coat the blood films is to extrude a large drop of durofix on one end of the slide and to spread it rapidly over the film by a single sweep of a clean finger.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOL. XXXI. No. 2. JULY, 1937.

Proceedings of the **30th Annual General Meeting** of the Society held at Manson House, 26, Portland Place, London, W., on Thursday, 17th June, 1937, at 8.15 p.m.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1937.

The HON. SECRETARY presented the Annual Report, copies of which had been circulated.

Col. J. A. Sinton proposed the adoption of the Report. This was seconded by **Dr. J. W. Lindsay** and carried.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31ST MARCH, 1937.

Dr. Oswald Marriott, *Hon. Treasurer*, presented his Report together with the accounts and Balance Sheet certified by Messrs. W. B. Keen & Co. and approved by the Audit Committee.

The Treasurer read his Report, and in conclusion reminded the Meeting that the Society's income depended very largely on the number of Fellows' subscriptions.

Dr. C. C. Chesterman, in proposing its adoption, congratulated the Treasurer on his excellent Report and expressed the hope that all Fellows wherever they are stationed should help by inviting suitable persons to become Fellows of the Society.

Col. A. G. Biggam seconded the motion which was carried unanimously.

ELECTION OF AUDIT COMMITTEE.

From the Chair the **President** proposed that Dr. V. S. HODSON, D GARROW and Major J. A. CRUICKSHANK be re-elected members of th Committee. To this the meeting agreed.

RESULT OF BALLOT FOR PRESIDENT, TWO VICE-PRESIDENTS AND TWENTY COUNCILLORS FOR 1937-39.

The **President** announced that 574 Voting Papers had been retu Fellows and that the following had been elected :—

President :

Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.).

Vice-Presidents :

Sir S. RICKARD CHRISTOPHERS, *C.I.E.*, *O.B.E.*, F.R.S., Col. I.M.S
H. HAROLD SCOTT, *C.M.G.*, M.D., F.R.C.P., F.R.S.E., D.T.M.

Councillors :

D. B. BLACKLOCK, M.D., D.P.H., D.T.M., Professor.
C. C. CHESTERMAN, *O.B.E.*, M.D., B.S., M.R.C.P., D.T.M. & H
S. F. DUDLEY, *O.B.E.*, M.D., F.R.C.P., D.P.H., D.T.M. & H., Rear-Admiral.
N. HAMILTON FAIRLEY, *O.B.E.*, M.D., D.Sc., F.R.C.P., D.T.M.
G. W. M. FINDLAY, *C.B.E.*, M.D., D.Sc.
E. D. W. GREIG, *C.I.E.*, M.D., D.Sc., F.R.C.P.E., Lt.-Col. I.M.S.
J. HEATLY-SPENCER, *C.B.E.*, M.D., B.S., F.R.C.P., D.T.M. & H. late R.A.M.C.
WILLIAM W. JAMIESON, M.D., F.R.C.P., D.P.H., Barrister-at-Law, Pro
W. P. MAC ARTHUR, *D.S.O.*, *O.B.E.*, K.H.P., M.D., F.R.C.P., I & H., Maj.-Gen., late R.A.M.C.
J. W. SCOTT MACFIE, D.Sc., M.B., Ch.B., D.T.M.
PHILIP MANSON-BAHR, *D.S.O.*, M.D., F.R.C.P., D.T.M. & H.
OSWALD MARRIOTT, M.D., B.S., M.R.C.P.
A. J. R. O'BRIEN, *C.M.G.*, *M.C.*, M.B., Ch.B., M.R.C.P., B.Hy.,
JOHN A. SINTON, *V.C.*, *O.B.E.*, M.D., D.Sc., B.Ch., D.P.H., D Lt.-Col. I.M.S.
Sir THOMAS STANTON, *K.C.M.G.*, M.D., D.Sc., F.R.C.P., D.P.H., D & H.
J. GORDON THOMSON, M.B., Ch.B., F.R.C.P., Professor.
C. M. WENYON, *C.M.G.*, *C.B.E.*, M.B., B.S., B.Sc., F.R.S.
F. NORMAN WHITE, *C.I.E.*, M.D., I.M.S. (ret.).
H. E. WHITTINGHAM, *C.B.E.*, M.B., Ch.B., F.R.C.P.E., D.P.H., D & H., Air Commodore R.A.F.
VINCENT B. WIGGLESWORTH, M.D., B.Ch.

The President (Sir ARTHUR BAGSHAWE): Before I hang this chain of gold about the neck of the new President I wish, as is customary, to make a few remarks about my two years' period of office. The Society pursues the even tenor of its way and, as you will gather from the Treasurer's Report and the Report of the Council, it has been a successful period. There is a considerable debt on Manson House but it is being steadily reduced, something over £2,000 having been paid off during my term of office. That has been helped by handsome donations from Mr. W. J. COURTAULD, and a second one from Mrs. CHALMERS who has thus increased our indebtedness to her. As you know she founded the Chalmers Medal of the Society which is to be presented at this meeting.

INDUCTION OF THE NEW PRESIDENT.

I now turn to my successor: Colonel JAMES, who is known to all, is a very distinguished member of a distinguished service. He has been engaged in research in malaria for a great number of years, I think I am right in saying ever since the Malaria Commission of the Royal Society was formed at the end of the last century. He has been putting out valuable papers all this time. He has done good work on other subjects but he will be mainly known for his work on malaria for which he has had the hall-mark of Fellowship of the Royal Society.

The retiring PRESIDENT then invested Colonel JAMES with the President's Badge and Chain of Office.

The President (Lt.-Col. S. P. JAMES): Sir ARTHUR BAGSHAWE, ladies and gentlemen, I thank you very much indeed for doing me the honour of electing me President of this Society, and I thank Sir ARTHUR BAGSHAWE in particular for the kind words that he has just said about me. I am the fourth member of the Indian Medical Service who, towards the end of his career, has received this high honour at the hands of his contemporary fellow workers, and it is an honour to the service to which I belong as well as to myself. I will only add that during my two years' term of office I will try my best to do all that I can to promote the interest and welfare of our Society.

I believe that my first duty as President is to nominate a Vice-President to serve during the two years of my office, and with your permission I propose to nominate a brother officer of my service, namely, Col. C. A. GILL, I.M.S., whom you all know very well. Colonel GILL became a member of this Society as long ago as 1908. His scientific attainments and his practical work in India and Ceylon are so well known that I need not mention them, but I ought perhaps to say that at two periods of his career in India he served with excellent results as Local Secretary. I hope very much you will accept with pleasure Colonel GILL as Vice-President.

Colonel C. A. Gill thanked the President for the honour he had done him in nominating him a Vice-President, and also for the kind remarks he had made.

PRESENTATION OF CHALMERS MEDAL.

The President: My next duty as President is to present the Chalmers Medal which is awarded every two years to a worker of any nationality under 45 years of age in recognition of notable additions to knowledge in tropical medicine and hygiene. We are fortunate to-night in that Mrs. CHALMERS is here with us to take part in this presentation—and I hope very much that when the presentation comes to be made Mrs. CHALMERS will assist me to present it. On this occasion the medal has been awarded to Prof. R. M. GORDON, of the Sir Alfred Jones' Research Laboratories in Sierra Leone. An outstanding merit of Professor GORDON'S work is that it covers a very wide field, and that it has all been done under difficult conditions in the tropics in Brazil and Africa. Recently Dr. GORDON has made the important discovery that tropical typhus fever exists in West Africa. His paper on that subject with Dr. DAVEY was published last year in Liverpool, and the discovery is bound to have far-reaching results. Unfortunately Dr. GORDON is unable to be present to receive the medal in person to-night, but I understand that Professor WARRINGTON YORKE or Professor BLACKLOCK will do so; I have therefore much pleasure in asking one or both of them to come forward and receive the medal and transmit it to Dr. GORDON. Perhaps Mrs. CHALMERS will be so kind as to help me.

Prof. WARRINGTON YORKE advanced to receive the medal and **Mrs. Chalmers** handed it to him with the remark " I am very pleased that Dr. GORDON has won this medal. I have been reading about the various papers he has written, and I should like to write to him myself and tell him how glad I am."

Professor Warrington Yorke: Sir, Mrs. CHALMERS, ladies and gentlemen, it gave me a very great pleasure to learn that my colleague, Professor GORDON, had been awarded this medal by the Council of this Society. I very much regret that he is not here to receive it in person to-night, and I am sure that when he learns of the circumstances attached to this presentation he will regret it also. I feel that he will appreciate this recognition of his work very highly, and that he will esteem the medal not less highly when he learns that it has been presented in person by its donor, Mrs. CHALMERS; and that this medal of gold was handed to her for the purpose by the PRESIDENT with the silver tongue.

This concluded the business of the Annual General Meeting.

Proceedings of an **Ordinary Meeting** of the Society
held after the Annual General Meeting
at Manson House, 26, Portland Place, London, W.,
at 8.45 p.m., on Thursday, 17th June, 1937.
Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (retd.), *President*, in the Chair.

PAPERS.

BLACKWATER FEVER IN MACEDONIA.

BY
HENRY FOY, M.A.
AND
ATHENA KONDI, M.D.
*League of Nations Malaria Research Laboratory, Refugee Hospital, Salonika.**

During five years work in Greece some 450 cases of blackwater fever have been seen and investigated in the laboratory, and some 900 made available for statistical analysis.

These cases have been drawn from the Refugee and Municipal Hospitals in Salonika, the villages and towns in the Plains of Drama and Chrysoupolis, and along the northern coast of the Peloponessus near Patras.

A full statistical analysis of our figures, together with charts, tables and equations, is now in the course of preparation and will be published later. Below we are only attempting to outline a brief general summary of our findings.

In Table I and Fig. 1 are shown the number of blackwater fever cases that have entered the two hospitals of Salonika in the period of 1923-1936, together with other related data on malaria and total admissions.

That this table represents only a part of the blackwater fever picture in Greece may be gathered from the fact that in 1933 out of thirty-seven cases occurring in an area under our control in the Peloponessus (Aighion) only three

*Dorothea Simmons Research Funds.

We are indebted to the GREEK MINISTRY OF HEALTH and the ATHENS SCHOOL OF HYGIENE for innumerable facilities that have been granted to us, without which most of this work would have been impossible.

To Drs. BARBER, RICE and M. C. BALFOUR, of the International Health Division of the Rockefeller Foundation we are greatly indebted for data on spleen and blood indices for malaria from various centres in Greece under their control.

TABLE I.

YEARLY DISTRIBUTION OF BLACKWATER FEVER AND MALARIA.

SALONIKA, 1923-1936.

Year.	Total Admissions.	Malaria Admissions.	Non-malaria Admissions.	B.W.F. Admissions.	Malaria Deviation from 1645.	B.W.F. Deviation from 60.	$10,000 \times \frac{m}{a}$ Malaria per 10,000 Admissions.	$10,000 \times \frac{b}{a}$ B.W.F. per 10,000 Admissions.	Percentage of of Total Admissions.		B.W.F. as percentage of Malaria admissions
									Malaria.	B.W.F.	B.W.F.
1923	6,423	1,934	4,489	9	+289	—51	3,012	14·0	30·12	0·14	0·47
1924	7,383	2,226	5,157	24	+381	—36	3,016	32·5	30·16	0·33	1·07
1925	6,777	1,456	5,321	35	—189	—25	2,148	51·6	21·48	0·52	2·04
1926	8,181	1,880	6,301	74	+235	+14	2,296	90·4	22·98	0·90	3·94
1927	8,296	1,638	6,658	60	— 4	± 0	1,974	72·3	19·74	0·72	3·58
1928	8,675	1,755	6,920	47	+110	—13	2,025	54·2	20·23	0·54	2·68
1929	9,149	1,518	7,631	51	—127	— 9	1,660	55·8	16·60	0·56	3·36
1930	10,025	1,514	8,511	65	—131	+ 5	1,511	64·8	15·11	0·65	4·29
1931	11,496	1,903	9,593	80	+258	+20	1,656	69·6	16·55	0·69	4·20
1932	12,844	1,992	10,852	121	+347	+61	1,551	94·2	15·51	0·94	6·07
1933	12,217	1,071	11,146	55	—574	— 5	877	45·0	8·70	0·45	5·14
1934	12,229	1,070	11,159	35	—575	—25	875	28·6	8·75	0·28	3·27
1935	12,342	1,393	10,949	48	—252	—12	1,127	38·8	11·27	0·39	3·45
1936	11,461	1,676	9,785	144	+ 31	+84	1,713	125·6	14·60	1·13	8·60
Total	137,498	23,026	114,472	848					16·7	0·62	3·6

entered the local hospital. We think it probable that not more than one quarter or one-fifth of the total number of cases of blackwater fever occurring ever reach the hospital, and since the disease is not a notifiable one, no accurate estimate of the real number occurring can be made. Our opinion is, however, that blackwater fever is probably very much more prevalent in Greece than anywhere in the world.

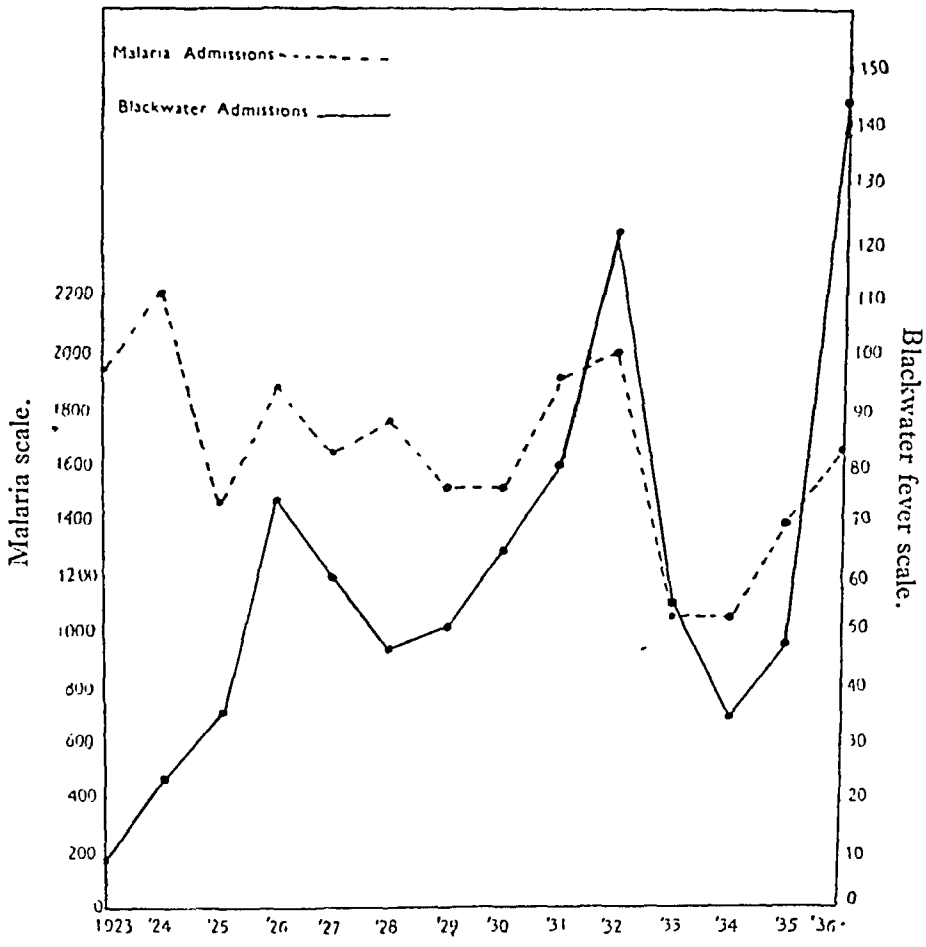


FIG. 1.—Malaria and blackwater fever admissions, 1923-36 (Salonika).

The low incidence of blackwater fever in 1923 and 1924 is perhaps not unrelated to the immigration of large numbers of refugees from areas in Asia Minor where malaria was either absent or of negligible importance. It is our opinion that this large immigration (variously estimated at between 700,000 and 1½ million) cannot be separated from the blackwater fever problem as it exists

in Macedonia, and the lack of correlation (see Fig. 1) between malaria and blackwater fever in 1923 and 1924 (when the refugees were arriving) is no doubt related to this.

According to STEPHENS, 1 or 2 years exposure to malaria is usually necessary before an individual develops his maximum susceptibility to blackwater fever. It is not surprising, therefore, that the malaria and blackwater fever of 1923 and 1924 lack the correlation that is evident in the later period, and in calculating the regression equation given below we feel we were justified in omitting the years 1923 and 1924.

Bearing in mind STEPHENS' hypothesis we might expect to find the number of blackwater fever cases (B) in any one year related to any or all of the following :—

(*i*) The number of malaria cases in the same year (M_0).
(*ii*) ,, ,, ,, ,, ,, 1 year previous (M_1).
(*iii*) ,, ,, ,, ,, ,, 2 years previous (M_2).

A regression equation was calculated expressing B in terms of M_0, M_1 and M_2 with the result that B was found to equal

$$0{\cdot}056M_0 - 0{\cdot}003M_1 - 0{\cdot}008M_2 - 7.$$

To determine which of these coefficients was significantly different from zero we used R. A. FISHER's tables of " t " to decide what probability there was of obtaining such a coefficient by chance from uncorrelated data. We found that

Coeff. M_0	$= 0{\cdot}056$	$P = 0{\cdot}04$
,, M_1	$= 0{\cdot}003$	$P = 0{\cdot}8$
,, M_2	$= 0{\cdot}008$	$P = 0{\cdot}7$

We are therefore justified in regarding B as independent of M_1 and M_2 and may construct an equation expressing B in terms of M_0.

When—

$$B = 0{\cdot}0554M_0 - 25{\cdot}6 \qquad P < 0{\cdot}02$$

and we must consider this coefficient significantly different from zero. The correlation coefficient between B and M_0 is 0·71, with a probability of obtaining such a figure by chance from uncorrelated data of less than 0·02.

It is clear from our regression equation :—That the number of blackwater fever cases in any one year is quite independent of the number of malaria cases either in the previous year, or the previous 2 years.

That in a population that has already been exposed to malaria for 1 or 2 years the number of blackwater fever cases is significantly correlated with the number of malaria cases of the same year.

That this correlation does not appear to hold in an unsensitised population, no matter how severe the malaria (cf. 1923 and 1924).

In addition to the relation with malaria, another fact emerges from a consideration of our figures, namely, that the susceptibility of the population of Macedonia to blackwater fever has been increasing with time, although somewhat irregularly. The 5 year averages of the blackwater fever cases graphed in Fig. 2 show quite a well-defined tendency to increase. The ratio $\frac{B}{M}$ graphed in Fig. 3.

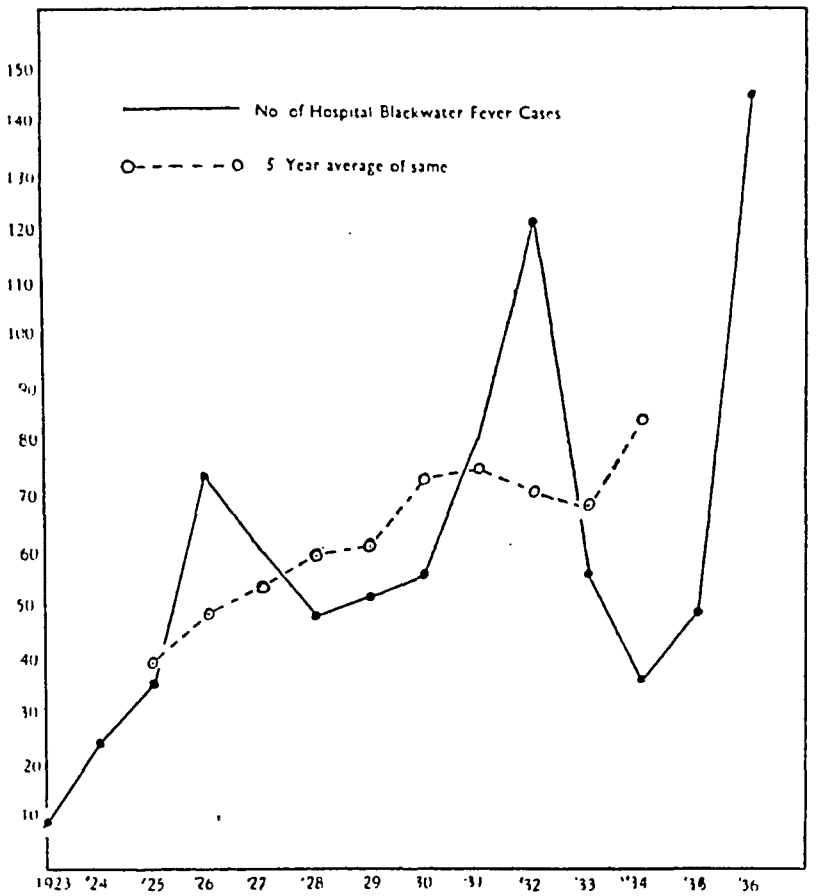


FIG. 2.—Five yearly averages of malaria and blackwater fever.

shows a similar, though irregular, tendency to rise. The graph of the 5 year averages of B and $\frac{B}{M}$ are not dissimilar, and their form suggests that the quantity graphed has risen from nearly zero in 1923, at first rapidly, but with a progressively diminishing rate of increase, and may be approaching an upper limiting value. Susceptibility, as measured by the ratio of $\frac{B}{M}$ may in future tend to oscillate about this upper limiting value, instead of increasing irregularly as it has done in the past.

Put in another way, it appears not unlikely that when blackwater fever has reached this upper limiting value then the "blackwater fever producing capacity" of malaria may become stable.

The monthly incidence of blackwater fever is given in Table II and Fig. 4, corrected for months of 30·44 days, from which it will be seen that the peak

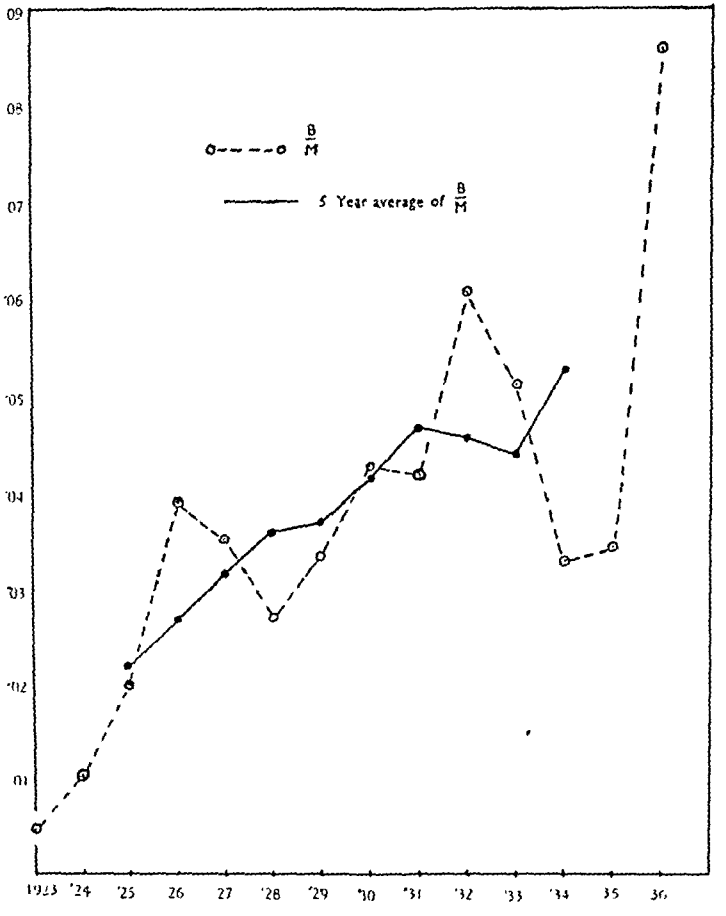


FIG. 3.—Ratio $\frac{B}{M}$ 1923-36.

for malaria admissions occurs in September (but is probably fairly evenly spread between August to mid-October), and that for blackwater fever in November. Thus the lag between malaria and blackwater fever is fully established. If the peak for blackwater fever is shifted forwards 2 months, the correlation

TABLE II.

MONTHLY DISTRIBUTION OF MALARIA AND BLACKWATER FEVER.

SALONIKA, 1923-1936.

Month.	Total Admissions.	Malaria Admissions.	B.W.F. Admissions.	Total Admissions corrected for period of 30·44 Days.	Malaria Admissions corrected for period of 30·44 Days.	B.W.F. corrected for period of 30·44 Days.	Percentage of Total Admissions that are Malaria.	Percentage of Total Admissions that are B.W.F.	Percentage of Malaria that are B.W.F.	Malaria Deviation from a mean of 1918·8.	B.W.F. Deviation from a mean of 74·0.
JAN.	10,581	1,234	65	10,389	1211·5	63·9	11·6	0·6	5·3	— 707	—10
FEB.	9,854	917	43	10,634	989·3	45·6	9·3	0·4	4·6	— 929	—29
MAR.	11,045	900	49	10,846	882·8	48·7	8·1	0·4	5·5	—1036	—26
APR.	10,343	785	36	10,493	794·5	36·5	7·5	0·4	4·6	—1124	—38
MAY	11,293	962	38	11,089	943·6	36·5	8·5	0·3	3·8	— 975	—38
JUNE	11,195	1,014	32	11,360	1028·9	32·3	9·3	0·3	3·2	— 890	—41
JULY	12,437	2,357	35	12,212	2313·4	32·3	18·9	0·2	1·4	+ 395	—41
AUG.	12,728	3,360	67	12,496	3299·7	63·9	24·4	0·5	1·9	+1381	—11
SEPT.	12,973	3,722	105	13,162	3774·5	106·5	28·6	0·8	2·8	+1856	+32
OCT.	12,669	3,287	127	12,441	3226·6	124·8	25·9	1·0	3·8	+1308	+50
NOV.	11,748	2,629	139	11,920	2666·5	140·0	22·3	1·2	5·2	+ 748	+66
DEC.	10,632	1,859	112	10,438	1823·3	109·6	17·4	1·0	6·0	— 95	+35
Total	137,498	23,026	848	137,482	22954·8	844·2	16·8	0·6	3·7		

between it and the malaria is almost perfect, the correlation coefficient being 0·93. (See Fig. 5.)

In connection with this seasonal distribution of blackwater fever it is of interest to note that the number of clinical cases of malaria entering the hospitals in September and October is very high, as also is the blood index. In the later part of October and early November the number of clinical cases shows a fall,

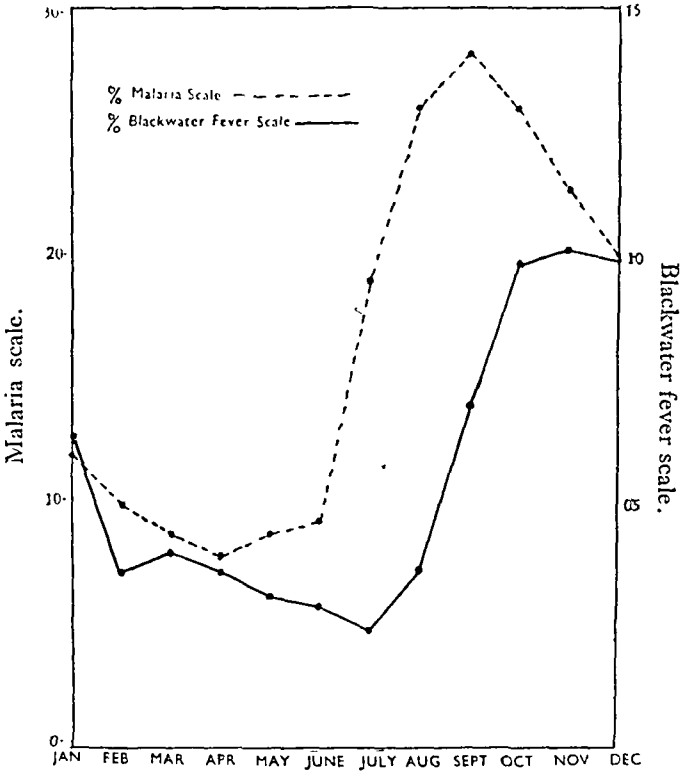


FIG. 4.—Monthly distribution of malaria and blackwater fever 1923-36 (corrected for month of 30·44 days).

but the blood index is almost as high as in the previous months, and the fall in the latter does not become steep until the end of November. It will be observed that the blackwater fever peak occurs in a period that appears to lie between an apparent malaria cure and a relapse, since the time of maximum malaria incidence is during August to mid-October, and the relapse period is in May. In Fig. 6, the blackwater fever admissions are plotted against the parasite indices for *vivax*, *falciparum* and *malariae*, from which it will be seen that the

2 monthly lag between the malaria and blackwater fever, evident when we plot the blackwater fever against clinical malaria admissions, has been reduced to 1 to 2 weeks when read against the *falciparum* curve. From these curves it will also be seen that there is a very close relation between the *malariae* peak and blackwater fever. We do not, however, attach much significance to this correlation of the *malariae* and blackwater fever peaks, because we have reason to believe that *malariae* is rarely or never associated with blackwater fever in Macedonia, as we shall show fully in our later analysis.

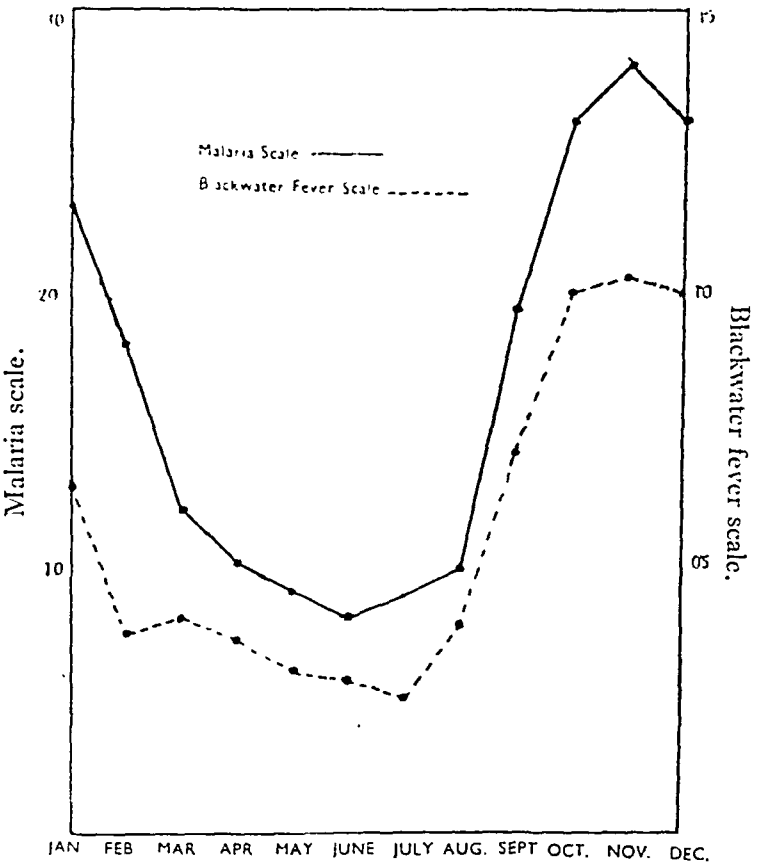


FIG. 5.—Monthly distribution of malaria and blackwater fever, with malaria shifted 2 months back.

Regarding the parasite rate in blackwater fever, we have found approximately 40 per cent. of our cases positive (360 cases examined). All the diagnoses were made on thick film smears, and in many cases confirmed by spleen puncture. We regarded as positive all cases that showed parasites any time during the course of their stay in hospital (which in some cases lasted 6 weeks). Our rate is no doubt lower than it otherwise would have been on account of the

large percentage of the cases that took quinine just prior to their attack. We do not consider that this quinine had a differential effect on *vivax* and *falciparum*, since from our work in the mental hospitals of Salonika we have found that *falciparum* in Macedonia is generally not more sensitive to quinine than *vivax*.

We would add that, in our opinion, parasite rates in blackwater fever have little meaning unless three factors are taken into consideration, viz. :—

(1) The amount and time of the last dose of quinine taken prior to the first passage of black urine.

(2) The magnitude and duration of the haemolysis as measured by red blood cell counts, and bilirubin estimations.

(3) The time that has elapsed between taking the blood smear and the first passage of black urine.

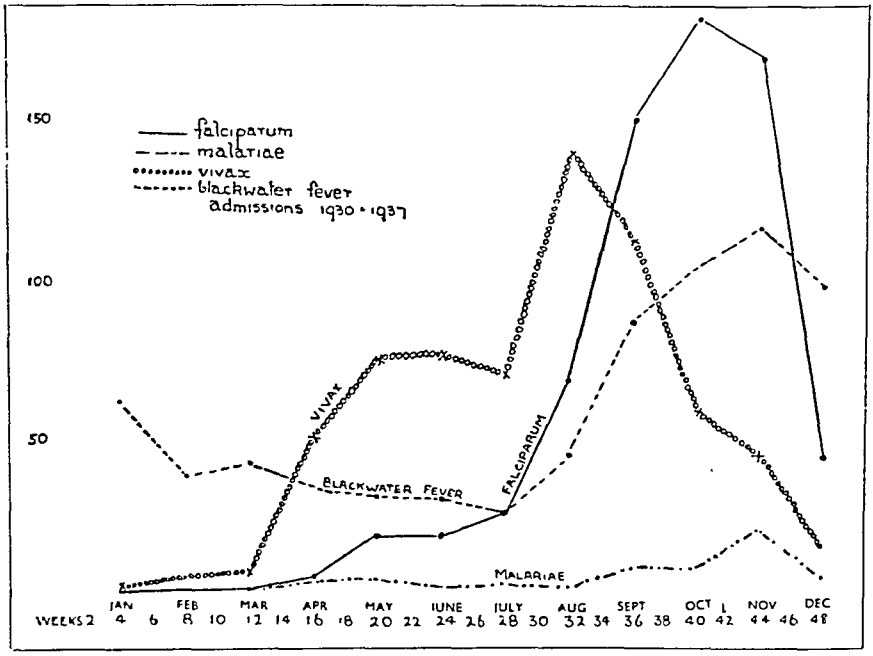


FIG. 6.—Monthly distribution of *vivax*, *falciparum* and *malariae* compared with blackwater fever admissions (partly from BALFOUR).

Of our positives 47 per cent. were *falciparum*; 33 per cent. *vivax*; 14 per cent. mixed *vivax* and *falciparum*; and 6 per cent. pigmented.

The greater percentage of *falciparum* should not necessarily be taken as meaning that *falciparum* is more potent in producing blackwater fever than is *vivax*, since we know that in Macedonia *falciparum* is about $1\frac{1}{2}$ times commoner

TABLE III.

AGE DISTRIBUTION.

Age Group.	No. of Total Admissions in Age Group.	No. of Malaria Admissions in Age Group.	No. of B.W.F. Admissions in Age Group.	Percentage of Total Admissions that are Malaria in Age Group.	Percentage of Total Admissions that are B.W.F. in Age Group.	Percentage of Malaria that are B.W.F. in Age Group.	School population.	
							Blood Index. Per cent.	Spleen Index. Per cent.
Years. 1– 5	5,365	1,165	26	21·71	0·48	2·23	43	68
6–10	3,675	1,185	52	32·25	1·42	4·38	48	81
11–15	4,859	1,729	57	35·56	1·17	3·29	36	85
16+	48,071	10,904	359	22·68	0·74	3·2	34	65
Totals	61,970	14,983	494	24·18	0·79	3·3		

in the general population than is *vivax*, consequently it is not surprising that a greater percentage of our blackwater fever positives showed *falciparum* parasites in their blood.

In a subsequent paper we shall discuss in detail the results of working regression equations expressing blackwater fever in terms of *falciparum*, *vivax* and *malariae*; for the present we would suggest that there appears to be little difference between the relative potency of *vivax* and *falciparum* so far as blackwater fever is concerned, with perhaps a slight chance in favour of *vivax* being the more blackwater fever producing. But since mixed infections are generally common in Macedonia it is not possible to rule out previous sensitization with *falciparum*, although in blackwater fever films *falciparum* may not be present.

FAMILY INCIDENCE.

As other workers, we have been struck with the large number of cases that occur in certain families. Out of 494 cases, 360 (= 73 per cent.) occurred in families one or more members of which had been previously attacked. The explanation sometimes given that such families often live in more malarious centres is by no means always found to hold.

AGE DISTRIBUTION.

In Table III the age distribution is given for the cases occurring in Macedonia. There is nothing particularly noteworthy in this; 0·97 per cent. occur in the age group below 16 years, and 0·75 per cent. above 16 years, which we may regard as practically identical.

DEATH RATE.

The death rate from blackwater fever in Greece is subject, as will be seen from Table IV, to very wide variations. On account of small numbers the earlier years cannot be regarded as very significant. It is interesting to note that (Table VII) the death rate is 22 per cent. in the age groups under 15 years where susceptibility is high, and 32 per cent. in the age group above 15 years where susceptibility is lower.

SEX RATIO.

In Table V is given the distribution of blackwater fever among the sexes. Our figures are in sharp contrast to those of Ross* who found that among Europeans in Southern Rhodesia blackwater fever was about six times more frequent among males than among females.

*Ross, G. R. (1932). Researches on blackwater fever in Southern Rhodesia. *Res. Mem. Lond. Sch. trop. Med.*, No. 6.

TABLE IV.

YEARLY FLUCTUATIONS IN DEATH RATE, 1923-1936.

(Refugee and Municipal Hospitals, Salonika.)

Year.	Number of Cases B.W.F.	Number of Deaths.	Percentage Fatality.
1923	9	4	44·40
1924	24	9	37·50
1925	35	15	42·86
1926	74	29	39·19
1927	60	16	26·66
1928	47	17	36·17
1929	51	12	23·50
1930	65	25	38·46
1931	80	25	31·25
1932	121	36	29·75
1933	55	11	20·00
1934	35	7	20·00
1935	48	7	14·50
1936	144	34	23·60
Total	848	247	29·10

FREQUENCY OF ATTACKS OF BLACKWATER FEVER.

One attack is much the most usual in Macedonia, but many go on to two or more. It is by no means uncommon in Greece, especially in the Peloponessus, to find individuals who have had as many as six to twelve attacks in the course of 10 or 20 years. The data relating to this are summarised in Table VI.

TABLE V.

SEX RATIO.

Year.	Total Admissions. (a) Male.	B.W.F. Admissions. (b) Male.	Percentage of Total Admissions that are B.W.F. (c) = $\frac{b}{a}$	Total Admissions. (d) Female.	B.W.F. Admissions. (e) Female.	Percentage of Total Admissions that are B.W.F. (f) = $\frac{e}{d}$	Ratio. (g) = $\frac{c}{f}$
1923	1,839	0	0	1,570	3	0·19	0
1924	2,411	5	0·21	2,080	6	0·28	0·75
1925	2,013	15	0·74	1,808	6	0·33	2·2
1926	2,276	34	1·5	2,361	18	0·76	1·97
1927	2,305	30	1·3	2,303	13	0·56	2·3
1928	2,696	18	0·66	2,396	19	0·79	0·83
1929	2,838	22	0·77	2,486	18	0·72	1·0
1930	2,722	33	1·2	2,569	26	1·0	1·2
1931	2,592	25	0·96	2,422	29	1·2	0·8
1932	2,653	38	1·4	2,760	38	1·4	1·0
1933	2,386	18	0·75	2,337	14	0·59	1·3
1934	2,345	12	0·51	2,406	11	0·45	1·1
1935	2,601	28	1·1	2,795	15	0·54	2·0
Totals	31,677	278	0·88	30,293	216	0·71	1·2

TABLE VI.

DISTRIBUTION OF NUMBER OF ATTACKS.

Number of Cases of B.W.F.	Number of Cases showing 1 Attack.	Percentage 1 Attack.	Number of Cases showing 2 Attacks.	Percentage 2 Attacks.	Number of Cases showing 3 Attacks.	Percentage 3 Attacks.	Number of Cases showing 4+ Attacks.	Percentage 4+ Attacks.	
196	124	63·2	32	16·3	16	8·2	24	12·2	Males.
165	102	61·8	48	29·1	15	9·1	0	0	Females.
361	226	62·6	80	23·1	31	8·6	24	6·6	Males and Females.

TABLE VII.

DEATH RATES AMONG ADULTS AND CHILDREN BY SEXES.

(13-year period 1923-1935.)

Age Group.	Number of Cases B.W.F.	Number of Deaths.	Percentage Mortality.	Number of Cases B.W.F.	Number of Deaths.	Percentage Mortality.	Number of Cases B.W.F.	Number of Deaths.	Percentage Mortality.
Year.	Male.			Female.			Male and Female.		
1–15	78	14	18·2	72	19	26·3	150	33	22·0
16+	252	89	35·3	191	55	29·2	443	144	32·5

Summary.

(1) A brief outline is given of some epidemiological findings regarding blackwater fever in Macedonia, a full report of which will be published later.

(2) Large numbers of cases of blackwater fever occur in Greece, probably not unrelated to the immigration of refugees from areas in Asia Minor where malaria was either absent or of negligible importance.

(3) Regression equations were calculated to define any relationship that existed between malaria and blackwater fever in M_0 M_1 and M_2 years with the result that a definite correlation was found to exist between the number of malaria cases in any year and the number of blackwater fever cases in the same year, *in an already sensitized population.*

(4) No correlation was established between the number of blackwater fever cases and the malaria cases occurring either 1 year or 2 years previously.

(5) No correlation existed between malaria and blackwater fever in an *unsensitized* population, no matter how severe the malaria.

(6) The susceptibility of the populations of Macedonia to blackwater fever has been steadily rising during the last 14 years, although somewhat irregularly. Susceptibility as measured by the ratio $\frac{B}{M}$ may in future tend to oscillate about an upper limiting value, instead of increasing irregularly as it has done in the past.

(7) The seasonal lag between the malaria and blackwater fever peaks is established; and the correlation found to be 0·93 when the blackwater fever peak is shifted forward 2 months.

(8) Blackwater fever appears to occur in a period that lies between an apparent malaria cure and relapse, as indicated by the seasonal distribution of malaria and blackwater fever, and the malaria relapse season in Macedonia.

(9) 40 per cent. of the cases of blackwater fever were found to be positive for malaria; 47 per cent. *falciparum*, 33 per cent. *vivax*, 14 per cent. mixed *falciparum* and *vivax*, 6 per cent. pigmented. This should not be taken to imply that *falciparum* is a " more potent producer " of blackwater fever than *vivax*, since *falciparum* is about $1\frac{1}{2}$ times more prevalent among the general population of Macedonia than is *vivax*. But parasite rates in blackwater fever should not be taken to have any great significance unless the following are known :—

(*i*) Time and amount of quinine taken prior to first passage of black water.

(*ii*) Magnitude and duration of the haemolytic process as measured by red blood cell counts, and bilirubin levels.

(*iii*) Time elapsed between first passage of black urine and taking blood film.

(10) Charts and tables are given showing age distribution, sex ratio, death rates, family incidence, frequency of attack, etc.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

PSEUDO-METHAEMOGLOBIN IN BLACKWATER FEVER AND ITS CLINICAL SIGNIFICANCE.*

BY

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AND

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In addition to oxyhaemoglobinaemia, methaemoglobin had been recorded in the plasma of blackwater fever patients by ARKWRIGHT and LEPPER (1918), YORKE, MURGATROYD and OWEN (1930), Ross (1932), and FAIRLEY and BROMFIELD (1934a). In the same year the last observers (1934b) recorded a new pigment closely allied to methaemoglobin in the plasma of a patient desperately ill with blackwater fever. The pigment was demonstrated in the circulating blood over a period of 10 days. Spectroscopically it resembled methaemoglobin, but failed to reduce with Stokes's reagent or ammonium sulphide (10 per cent.). It produced a chocolate coloured blood, a brown coloured plasma, was absent from washed corpuscles and failed to appear in the urine in demonstrable quantities. Specimens of plasma were sent to Prof. D. KEILIN who reported that, though the spectrum had the general appearance of methaemoglobin, the bands were shifted about 60 Ångström units towards the short wave end of the spectrum; it was easily reduced with sodium hydrosulphite ($Na_2S_2O_4$) and gave a typical haemochromogen (globin-protohaemochromogen), but apart from its spectroscopic similarity and its trivalent iron it had no properties of methaemoglobin when tested with alkali, H_2O_2, H_2S, azide, etc. Professor KEILIN regarded the pigment as entirely new, probably originating as a modification of methaemoglobin in which the globin portion of the molecule had been altered.

*The expenses of this investigation in Macedonia were met by a research grant from the London School of Hygiene and Tropical Medicine. We are greatly indebted to Mr. HENRY FOY, M.A. (Oxon.), for affording us such excellent facilities for laboratory investigation at the League of Nations Laboratory for Malarial Research, Salonika, and also to his assistant, Dr. A. KONDI, for invaluable assistance in the bedside study of patients. Dr. P. MANSON-BAHR has kindly placed his clinical material at our disposal in London, and our investigations here have been assisted by a grant from the Colonial Medical Fund.

About a year later the same pigment, which we have recently named pseudo-methaemoglobin (FAIRLEY, 1937), was again encountered. Owing to its importance the history of this second case along with the biochemical findings are recorded in some detail.

CASE 10. (London series.)

A male European from the Gold Coast, aged 48 years, was admitted to the Hospital for Tropical Diseases, London, under Dr. MANSON-BAHR, in a semi-conscious condition with haemoglobinuria of over 2 days' duration. Since Christmas, 1934, he had frequently suffered from malaria and in February, 1935, had developed blackwater fever while in West Africa. More recently he had been working in mines on the Gold Coast. During the voyage home he developed malaria and subsequently took quinine, 30 grains, each day.

Present Attack.—The patient arrived in England on 22.10.35. Five days before admission, *i.e.*, 6.11.35, he had felt ill and on 9.11.35 passed black-coloured urine.

10.11.35.—There was a severe rigor at noon lasting 4 hours, associated with pain in the right hypochondrium. Jaundice was present. He vomited everything he ate; the vomitus contained blood. Black urine was passed all day; later he became delirious.

11.11.35.—The patient arrived at hospital, semi-comatose with incontinence of urine and faeces. Temperature—101° F. Pulse—112. Respirations—32 per minute. The eyes were glazed, the skin was a pallid yellow, the conjunctivae bile-stained, the breathing stertorous, the tongue dry and furred and there were crepitations at both bases. The abdomen was distended and tender to palpation. The spleen was soft, tender and enlarged to the umbilicus (++). Catheterized—50 c.c. black urine. Faeces: black. Vomited dark green fluid. Blood pressure—S/D = 80/48. Veins so collapsed that it was difficult to insert needle. Plasma contained much oxyhaemoglobin and the new pigment, pseudo-methaemoglobin. R.B.C.'s = 910,000 per c.mm.; haemoglobin = 20 per cent. (Haldane); blood urea = 105 mg. per 100 c.c.; plasma CO_2 = 34·5 vols. per 100 c.c. Van den Bergh—direct reaction negative, indirect reaction positive (8 units). Urine—deep port-wine colour; acid in reaction; oxyhaemoglobin and methaemoglobin present; albumin = 0·55 per cent.; much urobilin; centrifuged deposit showed no casts, a few epithelial cells and amorphous deposit.

Treatment.—On 11.11.35, the day of admission, the patient was given 2 pints of sodium bicarbonate solution intravenously (150 grains to 1 pint), a blood transfusion of 750 c.c. of citrated blood and 500 c.c. of 5 per cent. glucose solution. Next day, *i.e.*, 12.11.35, he received 800 c.c. of citrated blood and 2 pints of 5 per cent. glucose solution intravenously. Atebrin, 0·1 gramme, was given thrice daily *per os* for 7 days, and a bicarbonate and citrate mixture prescribed three-hourly. On 13.11.35 another blood transfusion of 780 c.c. of citrated blood and 1 pint of 5 per cent. glucose solution were administered. Antiphlogistine was applied to the loins. Ferri et ammon. cit., grains 30, t.d.s.p.c., was commenced on 17.11.35 and continued throughout his illness. Ten days later, *i.e.*, 27.11.35, campolon injections (2 c.c. daily) were administered for 6 days. On 2.12.35 potassium iodide, grains 30 daily, was given and weekly injections of 0·3 gramme of stabilarsan and 1·5 c.c. of bismostab were instituted.

Progress Notes.—The immediate response to treatment was most satisfactory. By the following day, 12.11.35, the condition, though grave, had improved. The pulse was dicrotic and the blood pressure S/D = 80/48. R.B.C.'s = 1,090,000 per c.mm. Urine—amber coloured.

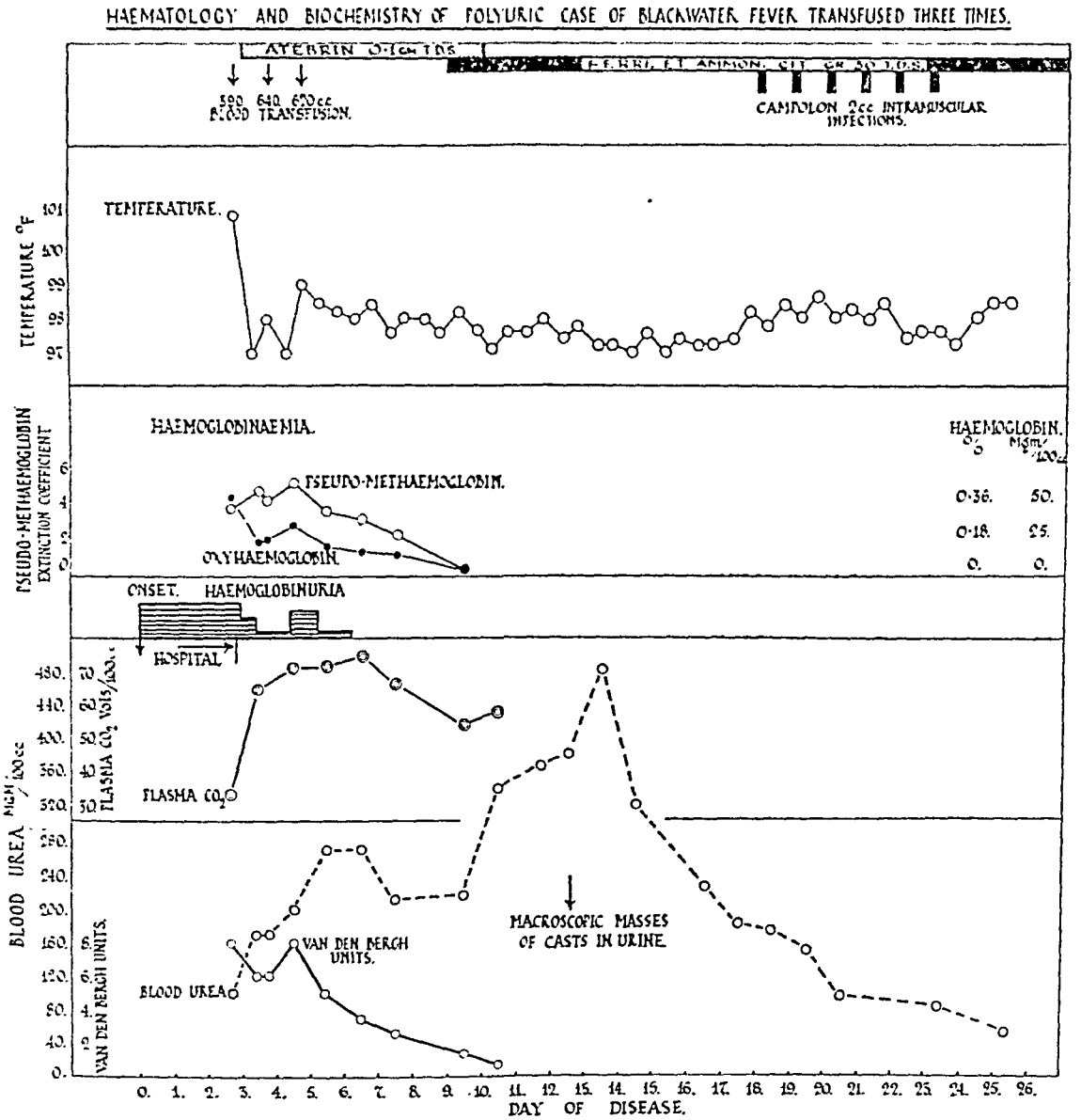
13.11.35.—Patient had a restless night with intermittent bouts of hiccough. After applying antiphlogistine to the loins 10 ounces of black urine were passed. Blood pressure—S/D = 104/50. R.B.C.'s = 1,425,000 per c.mm. Blood urea = 250 mg. per 100 c.c. The Wassermann and Kahn reactions were strongly positive (++).

14.11.35.—He spoke rationally, but bouts of hiccough were troublesome. Tongue was dry, yellow and furred. Spleen had decreased somewhat in size (+). Blood pressure—S/D = 110/64. R.B.C.'s = 1,875,000 per c.mm. Blood urea = 274 mg. per 100 c.c.

15.11.35.—Tongue was cleaner, but patient remained restless and drowsy, with bouts of hiccough. Blood pressure—S/D = 106/66. Blood urea = 276 mg. per 100 c.c.

16.11.35.—Abdominal tenderness had disappeared, spleen just palpable and hiccough less frequent. Urine was completely free from haemoglobin for first time. Blood urea = 210 mg. per 100 c.c. R.B.C.'s = 1,780,000 per c.mm.

19.11.35.—During the day patient became drowsy and vomited. Hiccough still troublesome. Blood urea = 348 mg. per 100 c.c. R.B.C.'s = 1,920,000 per c.mm.



CASE 10 (London series).

21.11.35.—Very drowsy after a restless night. Passed some small clots in urine. No vomiting or hiccough. Blood urea = 387 mg. per 100 c.c.

22.11.35.—Blood pressure—S/D = 108/66. Still passing small clots in urine and blood urea still rising (480 mg. per 100 c.c.). R.B.C.'s = 2,025,000 per c.mm.

23.11.35.—Improving. Blood pressure—S/D = 110/74. Tongue only slightly furred. No hiccoughing or vomiting for over 24 hours. Blood urea now falling (320 mg. per 100 c.c.).

25.11.35.—Still improving. Systolic haemic murmur. Edge of spleen just palpable. Blood pressure—S/D = 104/64. Blood urea = 227 mg. per 100 c.c.

27.11.35.—Improving steadily. Still passing urine with clots containing casts. Blood pressure—S/D = 104/66. Blood urea = 176 mg. per 100 c.c. R.B.C.'s = 2,235,000 per c.mm.; haemoglobin = 36 per cent.

4.12.35.—Greatly improved. Blood pressure—S/D = 120/80. Haemic murmur disappeared. Spleen not palpable. Blood urea almost normal (51 mg. per 100 c.c.).

9.12.35.—Improvement maintained. Urine normal. R.B.C.'s = 3,580,000 per c.mm.; haemoglobin = 54 per cent.; colour index = 0·8.

16.12.35.—Sitting out of bed for few days.

2.1.36.—R.B.C.'s = 4,530,000 per c.mm.; haemoglobin = 80 per cent.; colour index = 0·9. Blood urea = 24 mg. per 100 c.c.

3.1.36.—Left hospital.

COMMENT.

The rapid increase in the red cells following blood transfusion on 3 consecutive days was striking, the count rising from 910,000 per c.mm. (11.11.35) to 1,875,000 c.mm. (14.11.35) within 3 days. A maximal reticulocyte response of 18·4 per cent. occurred 10 days after the haemoglobinuria had commenced. Despite the reticulocyte response and the treatment adopted, blood regeneration was sluggish. The blood counts, however, gradually improved and on discharge from hospital on 2.1.36, *i.e.*, 52 days after onset, the R.B.C.'s = 4,530,000 per c.mm. and the haemoglobin = 80 per cent. (Haldane).

Renal Function.—Water excretion was good, a normal volume being passed throughout the illness. The urine was acid to litmus until 13.11.35 when the reaction became alkaline as a result of alkaline medication. Albuminuria was marked and haemoglobinuria of variable intensity persisted for 6 days. On the 3rd, 4th and 5th days true methaemoglobinuria coexisted with oxyhaemoglobinuria, but only oxyhaemoglobin was found in the urine on the 6th day. The new pigment—pseudo-methaemoglobin—was never demonstrable in the urine. On the Hartridge reversion spectroscope the α band coincided with that of artificially prepared methaemoglobin, while it was promptly dispersed by Stokes's reagent and dilute ammonium sulphide (10 per cent.).

Bile salts and bile pigments were never noted, but urobilinuria was present throughout this period. Casts were not numerous at first, but later small clots composed of mucus, leucocytes and casts were much in evidence. On 21.11.35 Col. F. P. MACKIE reported enormous numbers of cylindroids and casts. The casts were coarsely and finely granular and some were hyaline. A number of them contained globules of variable size, composed of hyaline material saturated with haemoglobin; superficially they resembled red blood corpuscles. They were identical with the casts blocking the tubules observed in sections of the kidneys of blackwater fever patients and malaria-infected monkeys developing haemoglobinuria (*P. knowlesi*). Their appearance in the urine in large numbers was followed within 48 hours by a dramatic drop in the blood urea, which

reached a maximum of 480 mg. per 100 c.c. on the 14th day (22.11.35) and subsequently rapidly decreased. A normal figure, however, was not attained until 3 weeks later (see Graph).

One other feature of note concerned the plasma CO_2 which on admission (11.11.35) had equalled 34·5 vols. per 100 c.c. The kidneys at this stage had obviously been damaged as a result of the initial excretion of oxyhaemoglobin. As in certain other cases of our series the decreased bicarbonate reserve resulted from an inability of the kidneys to secrete acid radicles adequately with consequent decrease in the alkaline reserve and a tendency to renal acidosis. Such a conclusion is in accord with the clinical findings for the patient was semi-conscious, very dyspnoeic and breathing stertorously at this stage of his illness. The rapid improvement following the injection of 2 pints of sodium bicarbonate (150 grains to 1 pint) and blood transfusion is depicted in the graph. Next day the plasma CO_2 had risen to 65·6 vols. per 100 c.c. and thereafter remained within normal limits.

Plasma Pigments.—The plasma contained both oxyhaemoglobin and the new pigment. Pseudo-methaemoglobin was demonstrated in the plasma from the 3rd to the 8th day, an extinction coefficient of 5 being reached on the 5th day, whereas the maximum oxyhaemoglobin concentration observed (51·8 mg. per 100 c.c.) was on the 3rd day (see Graph). These findings were also reflected in the colour of the plasma, which on the 3rd day was a deep brownish-red with the decrease in oxyhaemoglobin it became a deep golden brown.

The bilirubin content of the plasma was estimated daily, the indirect van den Bergh being 8 units on the 3rd and 5th days, after which it rapidly declined attaining a normal level on the 11th day (see Graph). The direct reaction was negative throughout.

MACEDONIAN CASES.

During the next 6 months no further opportunity was afforded of investigating blackwater fever patients in London, and after discussing the matter with Dr. C. M. WENYON and Professor W. W. JAMESON, arrangements were made with Mr. HENRY FOY, M.A. (OXON.), Director of the League of Nations Malarial Laboratory, Salonika, to carry out an investigation there. The remainder of this paper is based on data collected in Macedonia during November and December, 1936.

Sources of clinical material were the Refugee Hospital, which drains the population in the villages and rural districts surrounding Salonika, and the Municipal Hospital which deals with the townspeople of Salonika itself. Mr. HENRY FOY also arranged, through the Public Health Department, to be notified by telegram of blackwater fever patients within a radius of 80 kilometres of Salonika. In this way we were enabled to see some of the patients in country hospitals as well as in certain villages where blackwater fever was endemic

On these excursions, as well as in Salonikan hospitals, Dr. ATHENA KONDI acted as interpreter and rendered invaluable aid both in the collection of material and in the bedside study of patients.

Despite the fact that our investigations were mainly undertaken in December, when the incidence of blackwater fever begins to decline, it was impossible not to be impressed with the large number of patients who presented haemoglobinuria as a predominant feature of their illness, and with the unique potentialities of Macedonia for the study of this disease.

Some twenty-five cases were personally examined in a period of 6 weeks and, of these, fourteen were specially investigated from the viewpoint of the plasma and urinary blood pigments. It is with this group that the present paper mainly deals.

Particular attention was directed to other possible causes of intravascular haemolysis and atypical cases received special study. Bean haemoglobinuria was never met with; this, however, was not unexpected, as it does not occur anywhere in Greece during the winter months.

In our series all the patients were subjects of chronic malaria and had been in the habit of taking tablets of quinine sulphate for their fever. All, with one exception, had had quinine and plasmoquine by the oral or parenteral route or both within 24 hours of developing blackwater. In the exceptional case (Case VII) the patient was emphatic that he had received no anti-malarial drugs for 15 clear days before haemoglobinuria set in. Detailed information is available in the case histories published at the end of this paper [Appendix I.]

SCOPE OF LABORATORY INVESTIGATIONS.

Special care was taken in collecting the blood in tubes containing fluid oxalate in order to minimise plasmolysis. It was, however, rarely possible to collect specimens under paraffin, and we, therefore, adopted a slightly higher standard than formerly, namely, that the plasma haemoglobin should exceed a concentration of 20 mg. per 100 c.c. before it could be regarded as necessarily significant of true haemoglobinaemia. The technique adopted in quantitative estimations was a spectroscopic dilution method similar to that used by FAIRLEY and BROMFIELD (1933), and depended on the determination of the extinction coefficient of the α band of the different pigments under investigation. Multiple observations on consecutive days were made when possible and included haematological and biochemical investigations of the blood, and biochemical and cytological investigations of the urine. The blood urea and the plasma bilirubin were estimated as a routine. In addition both the plasma and urine were investigated for various blood pigments, and where an α band was noted in the red portion of the spectrum the specimen was examined on a Hartridge reversion spectroscope set against artificially produced methaemoglobin, and,

if it was considered necessary, sulphaemoglobin* as well. The methods we have used in the routine differentiation of blood pigments presenting an α band in the red portion of the spectrum are epitomized in Table I.

TABLE I.

THE METHODS USED IN THE ROUTINE DIFFERENTIATION OF CERTAIN BLOOD PIGMENTS* PRESENTING AN ALPHA BAND IN THE RED PORTION OF THE SPECTRUM.

Test.	Methaemoglobin.	Pseudo-methaemoglobin (New Pigment).	Sulphaemoglobin.
Alpha absorption band (Ångström units)	6,300	6,220—6,240	6,190
Stokes's reagent	Alpha band dispersed	Nil	Nil
Ammonium sulphide (10 per cent.)	Alpha band dispersed	Nil	Nil
Hydrogen peroxide (10 vols.)	Alpha band dispersed	Nil	Alpha band dispersed
Sodium hydrosulphite ($Na_2S_2O_4$)	Alpha band dispersed	Alpha band dispersed	Nil

*All these pigments produce a brown coloured plasma.

It will be noted that apart from the different position of the α absorption band in the spectrum of these pigments, their behaviour to certain chemical reagents varies. Thus, treatment with freshly prepared Stokes's reagent, ammonium sulphide (10 per cent.) and hydrogen peroxide (10 vols.) disperses the α band of methaemoglobin, but not of pseudo-methaemoglobin. Sodium hydrosulphite ($Na_2S_2O_4$), on the other hand, fails to disperse the α band of sulphaemoglobin, though it does completely disperse it in the case of the other two pigments. On reduction with sodium hydrosulphite, solutions of pseudo-methaemoglobin change to a cherry red colour due to the formation of haemochromogen; solutions of methaemoglobin change to a purple red owing to the production of reduced haemoglobin.

*KEILIN has recently shown that Hoppe-Seyler's supho-derivative of haemoglobin known as sulphaemoglobin is of undetermined nature and is not a simple reversible combination formed of H_2S with either haemoglobin or methaemoglobin. (*Proc. R. Soc.* 1933, B. vol, 113, p. 393.)

BLOOD PIGMENTS IN THE PLASMA OF BLACKWATER FEVER CASES.

The results obtained in the fourteen cases of blackwater fever specially investigated from this viewpoint are epitomized below.

TABLE II.

BLOOD PIGMENTS IN THE PLASMA OF BLACKWATER FEVER CASES.

Total Cases.	Oxyhaemoglobin.	Oxyhaemoglobin and pseudo-methaemoglobin.	Pseudo-methaemoglobin.	Methaemoglobin.
14	2	10	2	0

In the two instances (Cases IV and V) where oxyhaemoglobin alone was found in the plasma, both were children, and in both the haemoglobinuria was mild and of less than 24 hours' duration.

The two patients, whose plasma contained pseudo-methaemoglobin alone, were investigated in the post-haemoglobinuric stage and both were fatal. Biochemical data may be studied in Table III.

TABLE III.

BIOCHEMICAL OBSERVATIONS ON TWO FATAL CASES SEEN IN THE POST-HAEMOGLOBINURIC STAGE WHEN ONLY PSEUDO-METHAEMOGLOBIN WAS PRESENT IN THE PLASMA.

Case Number.	Day of Disease.	Blood Plasma. Colour.	Oxy-haemoglobin (mg. per 100 c.c.)	Pseudo-methaemo-globin (Extinction coefficient).	Bilirubin (van den Bergh units).	Blood Urea.
6	5th	Golden brown	Nil	3		169
	6th	Yellowish brown	Nil	1	9·6	224
	7th	Pale yellowish brown	Nil	Nil	2·8	170
	9th	Dark golden brown	Trace	1	6·0	160
	10th	„ „	Trace	1	4·5	263
8	3rd	Dark brown	Nil	1	15·0	253
	4th	„	Nil	Slight trace	9·5	297
	5th	Light brown	Nil	Nil	5·0	113
	7th	Yellow	Nil	Nil	4·0	196
	9th	„	Nil	Nil	1·0	333

The urine contained neither oxyhaemoglobin nor methaemoglobin. Urobilin was present.

One patient (Case VIII) died with anuria and renal acidosis on the 10th day. The plasma was not examined until the 3rd day when pseudo-methaemoglobin was found in small amounts (extinction coefficient = 1). A trace was present in a column 1 inch thick on the 4th day, but in no subsequent specimens. The other patient (Case VI) died from progressive anaemia and anoxaemia with adequately functioning kidneys on the 10th day of illness. Examination of the plasma on the 5th day revealed a considerable quantity of pseudo-methaemoglobin (extinction coefficient = 3), and small quantities of the same pigment were found on the 6th, 9th and 10th days (extinction coefficient = 1). Rigors, in the absence of demonstrable malaria parasites, occurred on the 8th, 9th and 10th days associated with increasing anaemia. The reappearance of pseudo-methaemoglobin in the plasma and the rise of its bilirubin content unassociated with demonstrable oxyhaemoglobin in the urine suggested that intravascular haemolysis had recurred, but that owing to the transient nature of the haemolysis and the rapid formation of pseudo-methaemoglobin in the blood stream, oxyhaemoglobin was appearing in the urine either in a quantity too small to be demonstrated, or not at all. Cases of this type with good water excretion, pseudo-methaemoglobinaemia and increasing anaemia are important to recognize since they belong to a group in which life can be definitely saved by repeated blood transfusion. These findings also explain one type of post-haemoglobinuric fever, associated with rigors in the absence of malaria parasites, which previously had not been understood. (*Vide* Appendix I, Case VI.)

In the remaining ten patients both oxyhaemoglobin and pseudo-methaemoglobin were found in the plasma. The urine contained oxyhaemoglobin in all instances and in nine out of the ten cases methaemoglobin as well. Details concerning the case histories may be consulted in Appendix I and biochemical data are epitomised on pp. 148-149.

The amount of haemoglobin present in the plasma of this series at different times varied from a minimum of 23·1 mg. to a maximum of 522 mg. per 100 c.c. The latter figure corresponds to 3·77 per cent. (Haldane scale). It is not at present possible to express the amount of pseudo-methaemoglobin quantitatively except in terms of the extinction coefficient, but using this method values of from 1·0 to 5·5 were recorded. In this regard it is well to remember that the absence of demonstrable pseudo-methaemoglobin in mild cases may have been due to its spectroscopic insensitiveness. Allied pigments which produce brown plasma and contain an α band in the red portion of the spectrum approximating to that of pseudo-methaemoglobin show poor relative intensities of their absorption bands. Thus, BLOEM (1933) found that the extinction coefficient for sulphaemoglobin was ten times more, and for methaemoglobin was nine times more, than for oxyhaemoglobin. These pigments therefore have to be present in considerable quantity in the plasma before they are demonstrable by direct spectroscopy, and the available data indicate that this is also the case with pseudo-methaemoglobin.

TABLE IV.

BIOCHEMICAL DATA ON BLACKWATER FEVER CASES IN MACEDONIA.

Case Number.	Day of Observation.	Plasma.					Urine.		
		Colour.	Oxyhaemoglobin (mg. per 100 c.c.).	Pseudomethaemoglobin (Extinction coefficient).	Haemobilirubin (van den Bergh units).	Blood Urea.	Oxyhaemoglobin (mg. per 100 c.c.).	Methaemoglobin (mg. per 100 c.c.).	Urobilin.
1	1st		+	Nil			+	Nil	
	2nd	Cherry red	+	1	10	164	+	Nil	
	3rd	Golden brown	Nil	3		190	Nil	Nil	+
7	1st	Deep red	+	1	17·6	69	+	+	
	2nd	Deep red	+	1	14	133	+	497·75	
	3rd	Light brown	Nil	Nil	7	187	Nil	Nil	
	4th	Light yellow	Nil	Nil	1·5	208	Nil	Nil	Nil
	6th	Pale straw	Nil	Nil	0·2	294	—	—	
15	2nd	Reddish brown	64·7	Nil	10·5	40	142	226·25	+
	3rd	Brownish yellow	69·3	1	8	48	121	452·5	+
	4th	Light brown	Nil	Nil	7·5	55	15·8	Nil	+
	5th	Pale yellow	Nil	Nil	1·5	40	Nil	Nil	
17*	3rd	Dark brown	60·0	3·5	33	88	168·3	226·25	+
	4th	Dark brown	23·1	5	38	143	15·8	Nil	+

19	2nd	Dark brown	55·4	3	19	43	157·8	271·5	+
	3rd						205	271·5	+
22*	4th	Dark red	522·1	3	11	155	89·4	454	+
	5th	Dark red	346·5	4	11	253	100	316·75	+
	6th	Almost black	92·4	5·5	10	187	23·6	90·5	+
23	2nd	Dark red	124·7	2	10	86	284	181·0	+
	3rd	Dark red	83·2	3	16	82	278·8	362	+
	4th	Golden brown	23·1	2·5	5·5	168	Nil	Nil	+
	5th	Light brown	Nil	Nil	1·5	138	Nil	Nil	+
	6th	Pale yellow	Nil	Nil	0·5	81			
24	2nd	Deep red	170·9	1·5	16	56	189·4	364·5	+
	3rd	Golden brown	60·0	2·0	11	99	142	Nil	+
	4th	Light brown	23·1	1·5	2	81	Nil	Nil	+
	5th	Pale yellow	Nil	Nil	1·75	51			
27	1st	Dark reddish brown	55·4	3·5	5	80	42·1	Nil	+
	2nd	Yellowish brown	20·8	1·5	2·75	55	Nil	Nil	+
28*	3rd						121·0	905	+
	4th	Golden brown	37·0	3	5	244	21·0	226	+

+ = positive. Nil = negative. * = fatal case

The time incidence in the appearance of pseudo-methaemoglobin is a matter of importance and from previous quantitative observations on our London cases based on the extinction coefficient of what, at that time, was erroneously thought to be methaemoglobin, as well as from quantitative observations on Macedonian cases, there can be little doubt that pseudo-methaemoglobin is formed from extra-corpuscular haemoglobin. The results of *in vitro* experiments entirely support this view. Though pseudo-methaemoglobin may occur in the plasma in association with oxyhaemoglobin as early as 4 hours after the reputed onset of haemoglobinuria, its appearance is sometimes delayed until the 2nd day. Quantitative observations indicate that its maximum concentration is attained later than that of oxyhaemoglobin, while not infrequently in fatal cases its concentration progressively rises until death, whereas that of oxyhaemoglobin falls. Graphs of such cases well illustrate the production of the new pigment at the expense of extracorpuscular haemoglobin. Furthermore, in patients examined during the post-haemoglobinuric stage where pseudo-methaemoglobin is alone recorded in the plasma, one daily examination of the urine is not sufficient. Investigation of each specimen of urine and examination of the plasma every few hours may be necessary to detect the transient recurrent haemolyses which are responsible for a pseudo-methaemoglobinaemia persisting over several days.

Repeated attempts were made to demonstrate pseudo-methaemoglobin within the corpuscles. For this purpose oxalated blood from blackwater fever cases was centrifuged, the supernatant plasma pipetted off and the corpuscles subsequently washed in isotonic saline and centrifuged. Suspensions of such corpuscles were invariably bright red in colour. When the packed corpuscles were subsequently lysed with distilled water, centrifuged and the supernatant reddish fluid examined for blood pigments, oxyhaemoglobin but never pseudo-methaemoglobin was found.

BLOOD PIGMENTS IN THE URINE IN BLACKWATER FEVER.

The pigments of special interest which have been described as occurring in the urine of blackwater fever, include oxyhaemoglobin, methaemoglobin, urobilin and a brown pigment, demonstrable in the centrifugalized deposit, which is generally regarded as acid haematin. With this last pigment, we do not at the moment intend to deal, except to point out that it has not the solubilities of artificially produced acid haematin and is difficult or impossible to demonstrate spectroscopically.

In the Macedonian cases, daily observations were made on the biochemical and cytological changes in the urine whenever possible, and data regarding the different blood pigments are summarized in Table V.

It will be seen that urobilinuria was invariably present at some stage of the disease. Oxyhaemoglobin and true methaemoglobin occurred in ten cases, and oxyhaemoglobin unassociated with methaemoglobin in two others. The

remaining two cases were examined in the post-haemoglobinuric stage, when only urobilin was present. Had they been investigated earlier, oxyhaemoglobin or methaemoglobin or both would undoubtedly have been found. It is interesting to note that in both these cases pseudo-methaemoglobinaemia was demonstrated.

The quantities of blood pigment actually present varied considerably in different cases and at different times in the same case. The minimum and maximum values of oxyhaemoglobin observed varied from 15·8 mg. to 284 mg. per 100 c.c., and those of the minimum and maximum for methaemoglobin from 90·5 to 905 mg. per 100 c.c.

TABLE V.
BLOOD PIGMENTS IN THE URINE.

Total Cases.	Oxyhaemoglobin.	Oxyhaemoglobin and methaemoglobin.	Pseudo-methaemoglobin.	Urobilin.
14	2	10	0	14

Pseudo-methaemoglobin was never found in the urine despite the fact that it was persistently searched for. Invariably on the Hartridge reversion spectroscope the bands of urinary methaemoglobin synchronized with those of artificially prepared methaemoglobin: furthermore, the α band was readily dispersed by Stokes's reagent, ammonia, ammonium sulphide (10 per cent.) and hydrogen peroxide (10 vols).

As pseudo-methaemoglobin was never demonstrated, and as it was not destroyed when plasma containing it was incubated with sterile urine for 24 hours at 37° C., we feel satisfied in concluding that it is a non-threshold substance for the kidney.

In connection with the spectroscopical examination of blood pigments in urine two observations of some practical interest were made. Specimens put aside for collection at the hospitals were not infrequently found to have a strong ammoniacal odour due to ammoniacal fermentation. Such urines were dark red in colour, strongly alkaline to litmus, but failed to show the α band of methaemoglobin. On acidification with acetic acid, however, methaemoglobin appeared. HARTRIDGE (1920) found that alkaline methaemoglobin was formed from methaemoglobin at a pH of 8·2 to 9·0. According to HAUROWITZ (1924) the conversion of acid into alkaline methaemoglobin begins at pH 7, is 50 per cent. complete at pH 8·4 and complete at pH 10. In these ammoniacal urines it was therefore not surprising that only alkaline methaemoglobin was found. Secondly, we noted on three different occasions that on adding ammonium sulphide to urine containing methaemoglobin the α band disappeared only to be replaced by another band in the red portion of the spectrum corresponding to sulphaemoglobin. HARRISON (1930) in a case of gross methaemoglobinuria due to *Bacillus welchii* septicaemia noted the same phenomenon, and Professor SNAPPER in a

personal communication informed him that it was not very rare in cases of methaemoglobinuria to convert some of the methaemoglobin into sulphaemoglobin under these circumstances. The change may be effected by either 10 per cent. or concentrated ammonium sulphide.

The Origin of the Urinary Blood Pigment.—All observers now appear agreed that oxyhaemoglobinaemia is directly responsible for the oxyhaemoglobinuria, the physiological teaching being that the oxyhaemoglobin is filtered through the glomeruli whenever its concentration in the blood exceeds the renal threshold. Opinion in regard to the source of the urinary methaemoglobin has however been divided. Some have regarded the condition as arising as a " true " methaemoglobinuria secondary to methaemoglobinaemia, others as a " false " methaemoglobinuria in which the methaemoglobin was formed from oxyhaemoglobin in the renal tubules or bladder.

Our finding that the pigment in the plasma, described previously as methaemoglobin, is a new pigment—pseudo-methaemoglobin—which is not eliminated via the kidneys, settles the question. There can now be no reasonable doubt that the methaemoglobinuria is of " false " type.

Renal Dysfunction and Anuria.—YORKE and NAUSS (1911) worked out the mechanism of anuria in blackwater fever and stressed the importance of adequate water excretion in preventing blockage of the tubules by haemoglobin debris. BAKER and DODDS (1925), in experimental work on rabbits, found that a pH of 6·0 or less and a concentration of NaCl exceeding 1 per cent. in the tubules favoured the formation of methaemoglobin and ultimately of acid haematin which, in their opinion, largely constituted the precipitate blocking the lumina. If the urine was alkaline in reaction the oxyhaemoglobin remained unmodified and was excreted as such in the urine without damaging the kidneys.

ROSS (1932) generally confirmed the *in vitro* experiments of BAKER and DODDS, but pointed out that certain anomalies were occasionally found in blackwater fever in man, such as the presence of oxyhaemoglobin in urine with a pH of 5·5, and of a mixture of oxyhaemoglobin and methaemoglobin in specimens with a pH of 6·8. Such paradoxical results were attributed to the facts (1) that methaemoglobin was formed over a wider range of pH than was indicated by the *in vitro* experiments, and that an acid reaction was not invariably required; (2) that the time elapsing between the exit of urine from the tubule and the time of examining the specimen was very variable. Since every specimen examined was a mixture of urine that had entered the bladder at all intervals during the period since that organ was last evacuated, it was not surprising that oxyhaemoglobin was found even in specimens with a low pH.

Any severe intravascular haemolysis in man ordinarily leads to blockage of the lumina of a proportion of the collecting and other tubules and to degenerative changes in their lining epithelium. Our pathological and biochemical findings indicate that if a sufficient number of nephrons be involved oliguria, anuria, nitrogenous retention with a high blood urea, uraemia and renal acidosis

characterized by a low plasma bicarbonate, low serum calcium and high blood phosphorus follow. On the other hand, where the initial haemolysis has been smaller and renal damage less extensive, the increased urea content of the blood exerts its well-known diuretic effect and polyuria results; such cases rarely die from uraemia but may succumb from anaemia if the haemolysis continues or recurrent haemolyses supervene.

Quantitative studies by YORKE, MURGATROYD and OWEN (1930) indicate that not more than 10 per cent. of extracorpuscular haemoglobin is excreted in the urine, the remainder being presumably disposed of by the reticulo-endothelial cell system and liver. An obvious explanation of the limited renal excretion of haemoglobin is decreasing functional efficiency associated with oliguria and anuria, but this view fails to explain why the haemoglobin excretion is also limited in cases of polyuria where the water excretion is high. In this respect the production of pseudo-methaemoglobin from extracorpuscular oxyhaemoglobin must be of considerable importance. Being a non-threshold substance for the kidney its formation in the blood stream is, in effect, a conservative process since it decreases the amount of haemoglobin available for excretion by the kidneys.

THE PRODUCTION OF PSEUDO-METHAEMOGLOBIN IN VITRO.

The fact that methaemoglobin can be spontaneously formed from haemoglobin has been recognised ever since HOPPE-SEYLÉR described the former pigment in 1865. NEILL (1925) pointed out that the presence of oxygen appears necessary for the spontaneous formation of methaemoglobin in blood or in haemoglobin solutions either at ordinary temperatures or at 55° C. The rate of conversion was related to the temperature at which the system was incubated, and the process involved was considered possibly to be an auto-oxidation by molecular oxygen, the iron perhaps serving as a catalyst. NEILL made an extensive study of the oxidation-reduction reaction of haemoglobin and methaemoglobin and specially noted the effects of living organisms such as pneumococci and certain anaerobes and of sterile animal and plant tissues in the production of methaemoglobin *in vitro*. In all his experiments solutions of oxyhaemoglobin and not whole blood were used. It is now recognised as a result of the work of CONANT (1923), CONANT and FIESER (1924) and CONANT and SCOTT (1926) that for the formation of methaemoglobin it is necessary to have reduced haemoglobin in the presence of some oxidizing agent, and in pure haemoglobin solutions the O_2 in equilibration with oxyhaemoglobin acts in this fashion. A condition in which the haemoglobin is partly oxidized and partly reduced is most favourable to the spontaneous production of methaemoglobin in haemoglobin solutions.

Believing that pseudo-methaemoglobin was peculiar to blackwater fever we postulated that the plasma in this disease must contain some substance of metabolic origin responsible for its formation. In order to ascertain whether this was

so, undiluted oxalated plasma collected under sterile conditions was incubated with a concentrated solution of haemoglobin at 40° C. for 48 hours in the ratio of 4 vols. of plasma to 1 vol. of haemoglobin. Pseudo-methaemoglobin was invariably produced, but when normal plasma was used as a control we were surprised to find that it exerted an identical effect. In both instances, when the pigment was compared with artificially produced methaemoglobin on the Hartridge reversion spectroscope, the α band was found nearer the blue ends of the spectrum. Further, it was not dispersed by such reducing agents as freshly prepared Stokes's reagent, ammonium sulphide (10 per cent.) or ammonia, and hydrogen peroxide (10 vols.) left it unchanged. Sodium hydrosulphite, on the other hand, dispersed the α band completely.

Finally in spectrograms taken for us by Mr. H. Foy, the centre of the α band of the artificially produced pigment approximated to 6230 Å, whereas that of methaemoglobin was constantly about 6,300 Å.

Solutions of oxyhaemoglobin in the absence of plasma incubated for a similar period produced true methaemoglobin. Similarly, well washed red blood corpuscles when incubated with physiological saline for 4 days at 40° C., were found to contain intracorpuscular methaemoglobin. When oxalated whole blood was incubated for a similar period, pseudo-methaemoglobin was found free in the plasma, whereas the washed corpuscles contained true methaemoglobin.

These experiments have been repeatedly confirmed and amplified, and we have found that the reaction occurs on incubation at 37° C., 40° C., 45° C. and 50°C. The presence of fluoride in the system does not appear to inhibit the reaction and decomplemented serum can be substituted for plasma without materially affecting the production of pseudo-methaemoglobin.

These experiments prove that plasma or serum has the power of producing pseudo-methaemoglobin directly or indirectly from extracorpuscular haemoglobin and indicate that in severe intravascular haemolysis other than blackwater fever, such as incompatible transfusion* and paroxysmal haemoglobinuria, this new pigment and not methaemoglobin will be found.

Other experiments were performed *in vitro* with artificially prepared solutions of methaemoglobin and sulphaemoglobin with similar results. When a solution of methaemoglobin prepared by treating laked corpuscles with potassium ferricyanide was incubated with plasma at 40° C. for 48 hours pseudo-methaemoglobin was formed. Similarly, when a solution of sulphaemoglobin prepared by bubbling H_2S through oxyhaemoglobin was incubated with plasma at 40° C. for 48 hours, pseudo-methaemoglobin again was formed.

It is evident, therefore, that plasma possesses the power to convert solutions of both these pigments, as well as oxyhaemoglobin, either directly or indirectly into pseudo-methaemoglobin.

*This prediction has since proved correct. On 23.6.37, some 6 days after this paper was read, Dr. Janet Vaughan kindly sent us specimens of plasma and urine from a patient who had been transfused with incompatible blood. The plasma was a dark golden-brown colour and contained oxyhaemoglobin (46·2 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 2) and bilirubin (2·5 units). The urine, which was blackish-red, was acid to litmus and contained oxyhaemoglobin (84 mg. per 100 c.c.), methaemoglobin (724 mg. per 100 c.c.) and hyaline, granular and epithelial casts.

Since drafting this paper we have found that if haematin* in alkaline solution (1 per cent. sodium carbonate) be incubated at 40° C. for a short period (1 hour or less) with four times its volume of oxalated plasma, pseudo-methaemoglobin is formed. Similarly if pure haemin* in alkaline solution (1 per cent. sodium carbonate or distilled water containing a few drops of 10 per cent. sodium hydroxide) is added to a requisite amount of plasma, pseudo-methaemoglobin is immediately produced. Haematin or haeme is a recognized disintegration product of the haemoglobin pigments, and it may well be that there are two stages in the formation of pseudo-methaemoglobin in the body—firstly, the production of haematin or haeme from extra-corpuscular blood pigment, and secondly its combination with some protein or other nitrogen-containing constituent of the plasma, to form a haemochromogen the possible nature of which is being investigated. ANSON and MIRSKY (1925) showed that haemochromogen is not simply free reduced haeme but is a compound of haeme and a nitrogen group, and that the particular haemochromogen prepared from haemoglobin is a compound of globin and haeme. These conclusions have been confirmed by HILL and HOLDEN (1926), ROCHE (1929) and others. If, however, globin be absent from the haemin, haematin and plasma used in our experiments it is difficult to see how a globin-haemochromogen could be formed.

This view is supported by comparing on the Hartridge reversion spectroscope the haemochromogen formed from pseudo-methaemoglobin by the action of sodium hydrosulphite with that prepared from reduced haemoglobin by alkali. Both solutions are cherry-red but their spectra are dissimilar.

SUMMARY AND CONCLUSIONS.

1. A new pigment which we have named " pseudo-methaemoglobin " has been constantly demonstrated in the plasma of severe cases of blackwater fever. In the past, this pigment has been erroneously regarded as methaemoglobin by all observers.

2. Certain spectroscopical and chemical reactions suitable for the routine differentiations of pseudo-methaemoglobin, methaemoglobin and sulphaemoglobin are described.

3. Pseudo-methaemoglobin produces a brown plasma, is never found within the corpuscles and is formed from oxyhaemoglobin only after its liberation from the red cells. It cannot function as a respiratory pigment.

4. The new pigment never appears in the urine in demonstrable quantities, nor is it destroyed by incubation at 37° C. with sterile urine for 24 hours.

5. Methaemoglobinuria is common in blackwater fever, but the methaemoglobin is derived from oxyhaemoglobin in the tubules or bladder and not as a result of methaemoglobinaemia.

*The pure haemin was kindly given us by Professor D. KEILEN, F.R.S., and the haematin obtained from British Drug Houses.

6. The formation of pseudo-methaemoglobin evidently constitutes a stage in a process by which the body disposes of circulating extracorpuscular blood pigment; in consequence the appearance of pseudo-methaemoglobin is to be anticipated in any intravascular haemolysis of sufficient magnitude.

7. The conversion of free oxyhaemoglobin into pseudo-methaemoglobin or any product impermeable to the kidneys is entirely beneficial since the amount of haemoglobin available for excretion in the urine is decreased and the tendency to renal damage lessened.

8. Pseudo-methaemoglobin can be artificially produced by incubating a concentrated solution of oxyhaemoglobin at 37° to 50° C. for 24 to 48 hours in the presence of a sufficient quantity of sterile plasma.

9. Similarly, when an artificially produced solution of methaemoglobin or sulphaemoglobin is incubated with sterile plasma at 40° C. for 24 to 48 hours pseudo-methaemoglobin is produced.

10. Pseudo-methaemoglobin may also be produced *in vitro* by some constituent in the plasma reacting with alkaline solutions of pure haemin or haematin.

11. Since globin is presumably absent from both these substances as well as from plasma, the suggestion is made that pseudo-methaemoglobin is formed by a combination of haeme or haematin with some nitrogenous constituent of the plasma other than globin.

12. When pseudo-methaemoglobin is reduced by sodium hydrosulphite a haemochromogen is formed which differs spectroscopically from globin-haemochromogen.

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APPENDIX I.

CASE HISTORIES OF MACEDONIAN SERIES.*

CASE I.

Female, aged 70 years, a subject of chronic malaria, was examined some 12 hours from onset of haemoglobinuria. One month ago, she returned from the village of Korovello where she had been reinfected. She had been taking 9 grains of quinine daily in irregular fashion. No previous attacks; no family history of blackwater fever. On 24.11.36 she developed fever, and 7 grains of quinine bihydrochloride were injected intramuscularly at 8 a.m. At midnight, *i.e.*, 16 hours later, she passed blackwater.

25.11.36.—Yellow parchment-like skin. Temperature = 100·4° F. Apical systolic murmur. No demonstrable enlargement of spleen or liver. R.B.C.'s = 3,500,000 per c.mm. No malaria parasites. Blood group II (Moss). The plasma collected 12 hours after onset contained oxyhaemoglobin in fair quantity, but not pseudo-methaemoglobin. The urine was red, ammoniacal in odour, alkaline in reaction and contained oxyhaemoglobin, casts and triple phosphates. Volume in 24 hours = 1,000 c.c.

26.11.36.—Appreciable jaundice. Spleen just palpable. Temperature = 98° F. R.B.C.'s = 2,812,000 per c.mm. The plasma now contained pseudo-methaemoglobin as well as oxyhaemoglobin. The urinary findings were the same as on 25.11.36. Volume = 1,600 c.c. Van den Bergh—positive indirect reaction (10 units). Blood urea = 164 mg. per 100 c.c.

27.11.36.—Urine contained urobilin, but not oxyhaemoglobin. Volume = 1,000 c.c. Plasma—golden brown, contained only pseudo-methaemoglobin. R.B.C.'s = 2,643,000 per c.mm. Blood urea = 190 mg. per 100 c.c.

Progress Notes.—A maximal reticulocyte count of 6·9 per cent. was recorded on 1.12.36, some 7 days after onset. R.B.C.'s next day = 2,250,000 per c.mm. and the reticulocytes = 2·4 per cent. Though the haemoglobinuria only lasted 2 days and blood destruction was not intense, subsequent progress was slow and the haematological response inadequate. Some 7 weeks later the patient was still in a debilitated, anaemic condition from which recovery appeared doubtful.

COMMENT.—The presence in the plasma of oxyhaemoglobin alone 12 hours from onset, the appearance of pseudo-methaemoglobin on the 2nd day and its presence in increased quantity on the 3rd day at a time when oxyhaemoglobin had disappeared from both plasma and urine, constituted the chief findings of interest in this case. Throughout water excretion was satisfactory, no tendency to anuria or oliguria being evident. The alkalinity of the urine was due to ammoniacal fermentation in a dirty urinal. Under these circumstances, methaemoglobin if present, is generally converted into alkaline methaemoglobin as we found later, and in consequence no α band is evident in the red portion of the spectrum.

CASE IV.

Female, aged 5 years, was seen 10 hours after onset of haemoglobinuria, several bouts of which had occurred during the past month. For several days, she had been receiving one tablet of atebrin and one of plasmoquine and about midnight, 26.11.36, developed transient haemoglobinuria.

27.11.36.—Anaemic child with yellow skin discoloured by atebrin. No demonstrable enlargement of spleen or liver. The plasma contained oxyhaemoglobin. Urine: black

*In all instances these patients were treated by their Greek physicians who kindly permitted detailed investigations, as recorded, to be carried out.

colour, acid reaction, much albumin, no casts; oxyhaemoglobin and methaemoglobin both present. R.B.C.'s = 2,955,000 per c.mm. No malarial parasites. Urea = 88 mg. per 100 c.c.

28.11.36.—Urine contained urobilin, but no oxyhaemoglobin. Van den Bergh—indirect reaction positive (3·2 units). Urea = 35 mg. per 100 c.c.

COMMENT.—The child suffered from a mild haemoglobinuria from which she rapidly recovered, the haemoglobinuria lasting less than 24 hours. Transient cases of blackwater of this nature are common in children in Macedonia. Pseudo-methaemoglobin was not present in demonstrable quantity, probably because the oxyhaemoglobin concentration in the plasma was minimal and of transient duration.

CASE V.

Female, aged 10 years, subject of chronic malaria. Quinine intake irregular. On the preceding day (26.11.36), had quinine sulphate *per os* at 9 p.m. and, according to her mother, passed black water half-an-hour later.

27.11.36.—The patient was not seriously ill and presented a spleen enlarged to the umbilicus (++). Blood smears showed no malarial parasites. The plasma contained oxyhaemoglobin. The urine was reddish-brown, acid in reaction, contained oxyhaemoglobin, albumin and urobilin, but no methaemoglobin. The centrifuged deposit showed scanty leucocytes and epithelial cells, but no casts.

COMMENT.—This is another example of transient haemoglobinuria in a child subject to chronic malaria.

CASE VI.

Male, aged 40 years, a subject of chronic malaria, had suffered from fever, rigors and vomiting for a fortnight. No previous attack of blackwater fever and no family history. Took quinine irregularly. History vague. Admitted to the Refugee Hospital, Salonika, with blackwater fever on 26.11.36. As far as can be ascertained, haemoglobinuria commenced on or about 23.11.36.

27.11.36.—Skin and mucous membranes were pallid and icteroid. The spleen was enlarged to the umbilicus (++), the liver was palpable and there was coffee ground vomitus. There was also an eruption on the skin of the arm, neck and abdomen consisting of closely clustered petechial haemorrhages resembling somewhat the rash of typhus fever. Water excretion was good, *i.e.*, 1,750 c.c. in 24 hours, and by this time the urine (some 4 days after onset), was clear of blood pigment.

Laboratory Examination.—R.B.C.'s = 1,764,000 per c.mm. Pseudo-agglutination was marked and the sedimentation rate rapid. Blood group No. IV (Moss). The plasma was golden-brown in colour and contained much pseudo-methaemoglobin, but no oxyhaemoglobin. Solutions prepared from washed lysed corpuscles showed no band in the red portion of the spectrum. Blood urea = 169 mg. per 100 c.c. Urine: clear, alkaline in reaction, contained a trace of albumin, numerous granular and epithelial casts, and a few leucocytes.

Progress Notes.—On 28.11.36 patient improved. Vomiting ceased. Wide-spread petechial eruption still present. No retinal haemorrhages. Blood pressure — S/D = 110/55. R.B.C.'s = 1,775,000 per c.mm. Blood urea = 224 mg. per 100 c.c. Plasma contained a faint band in red portion of spectrum (pseudo-methaemoglobin). Bilirubin = 9·6 units. Urine — same as on 27.11.36 except that the reaction was now acid and casts less evident. Volume = 1,200 c.c. in 24 hours.

29.11.36.—Still improving. Rash fading. Plasma: pale yellowish-brown, no pseudo-methaemoglobin or oxyhaemoglobin. Bilirubin = 2·8 units. Blood urea = 170 mg. per 100 c.c. Urine: clear, acid in reaction, blood pigments—nil, urobilin—present, epithelial casts—numerous. Volume = 1,150 c.c.

30.11.36.—Cold feelings followed by rigor. Temperature = 104° F. Patient looked ill. Rash not evident. Urine : clear, slightly alkaline in reaction, blood pigments—nil. Volume = 1,500 c.c.

1.12.36.—Another rigor. Temperature = 103° F. R.B.C.'s = 1,400,000 per c.mm. ; reticulocytes = 6·9 per cent. No malaria parasites. Plasma—dark golden-brown, contained pseudo-methaemoglobin (extinction coefficient = 1), but only a trace of oxyhaemoglobin. Bilirubin = 6 units (indirect). Urine : clear, acid in reaction, blood pigments—nil, albumin—trace, urobilin and a few granular and epithelial casts present. Urea = 1·5 per cent. Volume = 1,450 c.c. Blood urea = 160 mg. per 100 c.c.

2.12.36.—Rigor again last night. Patient looked very ill. Tongue—dry and pale. Collapsed veins. Dehydration evident. Blood pressure—S/D = 104/56. R.B.C.'s = 1,050,000 per c.mm. ; reticulocytes = 14·8 per cent. Plasma—dark golden-brown, containing pseudo-methaemoglobin, but only a trace of oxyhaemoglobin. Bilirubin = 4·5 units. Blood urea = 263 mg. per 100 c.c. Later temperature rose to 102·2° F., and patient died at 10 p.m. The bladder contained plenty of clear urine at autopsy. Analysis showed an acid reaction, albumin—trace, blood pigments—nil, granular epithelial casts and leucocytes—nil. Urea = 1·65 per cent.

Autopsy.—(3.12.36.) Some 12 hours after death a limited postmortem examination was made. Lice were present on the hair, and trunk, but all evidence of petechial eruption had disappeared.

Morbid anatomy.—The liver showed perihepatitis with adhesions to the diaphragm to which the base of the right lung was also adherent. The liver was of normal consistency, and yellowish-brown on section. The gall bladder was distended with tarry, black bile, containing 2,600 units (or 1·3 per cent.) of bilirubin (van den Bergh reaction). No pigment stones present.

The spleen was a very enlarged, flabby organ with marked perisplenitis ; the splenic pulp was reddish in colour and semi-fluid in consistency.

The kidneys were pale in colour, the capsule stripped readily and the cortex was well differentiated. The bladder was distended with clear urine.

Microscopic Pathology.—The polygonal cells showed a mild degree of cloudy swelling. The Küpffer cells contained a minimum of malaria pigment, but considerable amounts of haemosiderin were present in the parenchyme cells.

Spleen.—The sections revealed lymphoid tissue interspersed with trabeculae composed of fibrous tissue. The Malpighian corpuscles were conspicuous by their absence. Malarial pigment was demonstrable in the endothelial cells and haemosiderin was also noted.

Kidneys.—The glomeruli were mostly healthy, but a few of the tufts were obliterated by fibrosis and there were some early retention cysts and increase in interstitial fibrous tissue. A few cortical arterioles were thrombosed and the coats of the larger arteries were somewhat thickened—evidences of chronic arteriosclerotic changes.

The epithelium of some groups of tubules showed toxic desquamation with fine pigmentation of the cells and an eosinophilic reaction. Only certain nephrons were involved. The collecting tubules were mostly unaffected, but a few were silted up with homogenous eosinophil-staining material. From a pathological viewpoint the kidneys could not be regarded as seriously involved. A granular pigment like haemosiderin, giving the Prussian blue reaction, was

noted within the cells of the tubules and their lumina and in the collecting tubules as well. Sometimes a diffuse blue staining occurred.

COMMENT.—This patient appeared to be progressing satisfactorily when exacerbation of fever with recurrent rigors and increasing anaemia culminated in death. Examination of both thick and thin blood films failed to reveal malaria parasites. The urine was clear and had been free from blood pigment for the last six days of his illness. Water excretion was highly satisfactory, the daily output varying from 1,150 to 1,750 c.c. Estimations of the urinary urea on the last 2 days preceding death equalled 1·5 and 1·65 per cent., while both granular and epithelial casts were scanty in the terminal phases of the illness. Furthermore, microscopic sections showed that blockage of the tubules with eosinophile granular debris and degenerative changes in the tubular epithelium were minimal, and not sufficient to interfere seriously with renal function.

The reappearance of pseudo-methaemoglobin and traces of oxyhaemoglobin in the plasma in the later phases of the illness in association with recurrent rigors but unassociated with haemoglobinuria is noteworthy. This suggested that haemolysis was occurring, but that at this late stage the plasma was converting extra-corpuscular oxyhaemoglobin sufficiently rapidly into pseudo-methaemoglobin to prevent the oxyhaemoglobin reaching a concentration sufficient to exceed the renal threshold or to appear in demonstrable quantity in the urine. Such a mechanism would explain the exacerbations of fever with rigors, the increased anaemia, the rise in bilirubin and urea and the absence of haemoglobinuria.

It also explains one baffling type of post-haemoglobinuric fever, associated with recurrent rigors and the absence of malarial parasites, which has never been understood. Another aspect of considerable practical importance in this connection concerns treatment. Death in this case was due to anaemia in the presence of adequately functioning kidneys, and a bone marrow which was showing a reasonable reticulocyte response—*i.e.*, 14·8 per cent. Blood transfusions, under circumstances such as these, are a life saving procedure, as illustrated in Case 7 (These TRANSACTIONS, 1934, **28**, 311) and Case 10 of our London series, and, when practicable, should be carried out.

CASE VII.

Male, a refugee from Bulgaria, aged 53 years, was subject to chronic malaria. He had taken no quinine for 15 days, but before that took 20 grains of quinine sulphate daily for some time. The day before (27.11.36), at 8 p.m., he started shivering; this lasted all night and at 5.30 a.m. he passed black water. His first attack was in 1927. There was no family history.

28.11.36.—The patient was examined 6½ hours after the onset of haemoglobinuria. Warm flushed skin with icteroid tinge. Temperature = 102·6° F. Spleen enlarged to umbilicus (++). Passing much dark red urine.

Laboratory Examination.—R.B.C.'s = 3,870,000 per c.mm. No parasites. Auto-agglutination marked. Rapid sedimentation rate. Group II. (Moss). Plasma—

a deep red colour—contained oxyhaemoglobin but only a small amount of pseudo-methaemoglobin (extinction coefficient = 1). Bilirubin = 17·6 units (indirect). Blood urea = 69 mg. per 100 c.c. Urine—deep red, alkaline in reaction (due to ammoniacal fermentation) much oxyhaemoglobin and also alkaline methaemoglobin as shown by the reappearance of the α band of true methaemoglobin on the addition of acetic acid. Volume = 1,600 c.c. in 24 hours. At 10 p.m. there was another shivering attack with vomiting lasting half-an-hour.

29.11.36.—Tongue was dry. Icteroid tinge to skin. Blood pressure—S/D=116/70. Urine—acid, contained large amount of oxyhaemoglobin, methaemoglobin, albumin, also leucocytes and casts. Volume 1,200 c.c. R.B.C.'s = 1,725,000 per c.mm. No parasites. Plasma—deep red colour, containing much oxyhaemoglobin and small quantity of pseudo-methaemoglobin (extinction coefficient = 1). Bilirubin = 14·0 units (indirect). Blood urea = 133 mg. per 100 c.c.

Progress Notes.—On 30.11.36 the urine cleared and never subsequently contained blood pigment. Bilirubin = 7·0 units (indirect). Blood urea = 187 mg. per 100 c.c.

1.12.36.—R.B.C.'s = 1,400,000 per c.mm. Bilirubin = 1·5 units. Price-Jones curve showed marked displacement to the right, a megalocytosis of 38·6 per cent., a mean average diameter of 8·238 microns, a coefficient of variation of 7·784 and a standard deviation of 0·649. Reticulocytes = 0·5 per cent. Haematocrit = 15·6. Corpuscular volume = 111·4 cubic microns. Patient well, reading papers. Spleen still ++ ; some tenderness and rigidity of right rectus over gall bladder.

3.12.36.—Median basilic vein thrombosed with induration of perivenous tissues. R.B.C.'s = 1,612,500 per c.mm. Reticulocytes = 2·1 per cent. Bilirubin = 0·2 units. Blood urea = 294 mg. per 100 c.c. Blood pressure = S/D = 120/72.

7.12.36.—R.B.C.'s = 1,756,000 per c.mm. Reticulocytes = 12·3 per cent. (maximum rise). Bilirubin = 1·2 units. Urea = 127 mg. per 100 c.c. Daily intramuscular injections of glucose (500 c.c.), which had been given since admission, were stopped. Subsequently the temperature became normal. Throughout this period the water secretion had been satisfactory, the daily volume varying from 1,150-1,700 c.c.

11.12.36.—R.B.C.'s = 2,250,000 per c.mm. Reticulocytes = 3·1 per cent.

14.12.36.—Bilirubin = 1·5 units. Urea = 62 mg. per 100 c.c.

24.12.36.—No further rise of temperature, but the patient was still anaemic and not making progress anticipated. The diet was very restricted, consisting mainly of rice water, orange and lemon juice and abundant fluids. R.B.C.'s = 2,460,000 per c.mm.

6.1.37.—Malaria relapse with intermittent fever. Gametocytes of *P. falciparum* demonstrated.

Injections of atebrin, 0·2 grammes daily from 8th to 11th. Temperature now normal. From 12th to 14th injections of plasmoquine, 0·3 gramme daily, were administered.

17.1.37.—Left hospital.

COMMENT.—It is noteworthy that haemoglobinuria developed in this patient in the absence of recent quinine treatment, no anti-malarial drug having been taken for 15 clear days before its onset. The plasma contained a small quantity of pseudo-methaemoglobin (extinction coefficient = 1) during the first 2 days, and during this period there was a significant oxyhaemoglobinaemia. The Price-Jones curve, taken on the 4th day, showed a well-marked megalocytic anaemia. A high corpuscular volume of 111·4 c. microns was also observed on the 4th day, while on the 10th day it equalled 105·4 c. microns. For reasons given elsewhere megalocytic anaemia of this degree is to be attributed mainly to dietary deficiency rather than to malaria or blackwater fever. A maximal reticulocyte response of 12·3 per cent. was noted on the 10th day, but blood regeneration was tardy—a fact attributable to the megaloblastic nature of the red marrow and the effects of latent malignant tertian malaria. Malarial fever supervened on

the 38th day of illness and malignant tertian paras
smears 2 days later. Atebrin treatment commenced 8
temperature became normal. The patient was discl
record of the final blood count or Price-Jones curve v

Case VIII.

Male, aged 62 years, a refugee from Constantinople,
He had suffered from chronic malaria throughout the sum
only when fever was actually present. There was no previou
of blackwater. On 30.11.36 he developed fever and took 6 gr
and evening, and before dawn on 1.12.36 he took plasmoqu
1 hour later, *i.e.*, 6 a.m., he passed red urine.

3.12.36.—Examination some 54 hours after onset showe
and tender. The spleen was palpable midway between the co
(+). The patient looked ill. The skin and conjunctivae we

Laboratory Investigations.—The urine was brown in co
much urobilin, leucocytes, epithelial cells and a few granula
pigment was not present. The plasma contained no significan
but pseudo-methaemoglobin was present in appreciable quantit
The α band in the red was not dispersed by Stokes's reage
ammonium sulphide ; it disappeared with strong ammoniu
reaction : direct—biphasic positive, indirect—positive (15 un
per 100 c.c. R.B.C.'s = 2,010,000 per c.mm. Rapid sedime
24·5. Mean corpuscular volume = 122·5 c. microns.

Treatment.—Some 600 c.c. of a 5 per cent. solution c
muscularly and subsequently daily injections of 300 to 600
Atebrin, 0·3 gramme, was given each day for 5 days.

4.12.36.—Hiccough present. Blood pressure—S/D =
R.B.C.'s = 1,900,000 per c.mm. Haematocrit = 20. C
c. microns. Reticulocytes = 0·1 per cent. Urine—same as o
per cent. Plasma—only a trace of pseudo-methaemoglobin i
Van den Bergh : direct—biphasic positive, indirect—positive
297 mg. per 100 c.c.

5.12.36.—Temperature = 102° F. Cough present.
which contained a small amount of albumin and urobilin,
showed no blood pigments. Van den Bergh : indirect—posit
113 mg. per 100 c.c. Pseudo-agglutinin still present. Haem
= 0·8 per cent.

7.12.36.—Patient vomiting. Tendency to retention of u
Dyspnoea without cyanosis noted. Temperature—subno
Pulse = 104 per minute. Van den Bergh : indirect—positi
196 mg. per 100 c.c.

9.12.36.—Condition much the same—dyspnoea suggest
333 mg. per 100 c.c. Van den Bergh : indirect—positive (1 un
Died with renal acidosis, anuria and broncho-pneumonia.

Comment.—When seen on the 3rd day after onse
urine contained neither oxyhaemoglobin nor methaen
showed pseudo-methaemoglobin, but no significant ox
the haemolytic process had ceased. The temperature f
likely. Bronchitic features, however, developed and ur

CASE XV.

Male, aged 39 years, had lived on the outskirts of Salonika for 8 years. There was no family history of blackwater and the present was the first attack. Malaria contracted in 1918 during the War, but he had not been troubled with it again until this summer. Each time he developed fever 1·5 grammes of quinine (23 grains) was injected intramuscularly. On 6.12.36, he had another malarial attack for which the usual injection was given. Fever recurred next day and the injection was repeated. On 8.12.36, there was no further rise of temperature, but another injection was given. This was repeated at 5.30 p.m. the following day (9.12.36). One hour later he had a rigor and passed black urine. Shivering lasted until midnight and haemoglobinuria has persisted since.

10.12.36.—The patient complained of headache, nausea and vomiting. Physical examination showed some degree of jaundice, pallor and yellow tinting of the mucous membranes and enlargement of the spleen.

Laboratory Investigations.—Urine was almost black in colour, acid in reaction and contained oxyhaemoglobin (142 mg. per 100 c.c.) and methaemoglobin (226 mg. per 100 c.c.), urobilin and albumin. Numerous epithelial and granular casts, pus cells and epithelial cells were present. The plasma contained oxyhaemoglobin (64·7 mg. per 100 c.c.). Van den Bergh : direct—negative, indirect—positive (10·5 units). Blood urea = 40 mg. per 100 c.c. R.B.C.'s = 3,000,000 per c.mm. Reticulocytes = 0·3 per cent. Haematocrit = 27·25. Corpuscular volume = 91·9 c. microns.

Treatment.—This included atebrin (0·3 gramme), 500 c.c. of 5 per cent. glucose intravenously on 10.12.36 and intravenous saline (600 c.c.) for the next 2 days. Daily injections of calcium gluconate were also given.

11.12.36.—R.B.C.'s = 2,184,000 per c.mm. Blood group IV (Moss). Reticulocytes = 0·2 per cent. Plasma now definitely contained pseudo-methaemoglobin (extinction coefficient = 1) and oxyhaemoglobin (69·3 mg. per 100 c.c.). Urine same findings as on preceding day, except that oxyhaemoglobin = 121 mg. per 100 c.c. and methaemoglobin = 452·5 mg. per 100 c.c. Bilirubin = 8·0 units (indirect). Blood urea = 48 mg. per 100 c.c.

12.12.36.—Plasma contained oxyhaemoglobin (13·9 mg. per 100 c.c.). Bilirubin = 7·5 units (indirect). Blood urea = 55 mg. per 100 c.c. Urine—morning specimen contained trace of oxyhaemoglobin (15·8 mg. per 100 c.c.) which had disappeared by 6 p.m. Urobilin still present in considerable amounts, also granular and epithelial casts. R.B.C.'s = 1,800,000 per c.mm. Haematocrit = 15·8. Mean corpuscular volume = 87·7 c. microns.

14.12.36.—Temperature normal for first time. Reticulocytes = 3·3 per cent.

16.12.36.—Still looked anaemic, and spleen enlarged to midway between umbilicus and costal margin (+). R.B.C.'s = 2,100,000 per c.mm. Reticulocytes = 8·3 per cent. Bone marrow showed erythroblastic response. Daily injections of plasmoquine were given from the 16th to 18th December. On the latter date, a reticulocytosis of 10·8 per cent. was observed.

COMMENT.—This was not a very severe case of blackwater. The appearance of pseudo-methaemoglobin in demonstrable quantity was delayed until the 3rd day when the concentration of oxyhaemoglobin equalled 69·3 mg. per 100 c.c. Next day the plasma was clear of blood pigments. A maximal reticulocytosis of 10·8 per cent. was noted on the 10th day. A satisfactory erythroblastic response was noted in the bone marrow smears, blood regeneration proceeded along normal lines and the patient was later discharged from hospital.

CASE XVII.

Female, aged 27 years, was born in Macedonia and had suffered from malaria for many years. This was her first attack of blackwater fever ; her father had died from it. She lived in Salonika, but had passed the summer in a Macedonian village where she was reinfected. She took quinine *per os* during attacks but on only one occasion did she receive an intramuscular injection of quinine. On 9.12.36 she felt ill and at 9 p.m. took 6 grains

of quinine. Fifteen hours later, *i.e.*, at noon on 10.12.36, she had a rigor, became feverish and passed blackwater which has persisted since. Admitted to Hospital at 5 p.m. Temperature 104·4° F. Pulse = 140. Anaemic and jaundiced. Urine—black, some 700 c.c. passed until midnight, *i.e.*, in 7 hours.

12.12.36.—Patient was examined at 1 p.m. She was very jaundiced, restless and troubled with severe bilious vomiting. The spleen was enlarged to midway between the umbilicus and the pelvic brim (+++) ; there was well-marked hepatomegaly and the upper abdominal muscles were tender and rigid over the liver. Blood pressure—S/D = 115/70. Later she had a rigor and at 6 p.m. was worse. The urine was still black in colour ; 1000 c.c. passed since midnight, *i.e.*, in the last 18 hours.

Laboratory Investigations.—R.B.C.'s = 1,265,000 per c.mm. Haematocrit = 13·0. Corpuscular volume = 102·8 c. microns. Blood group II (Moss). One gametocyte was found by Dr. A. KONDI in a thick blood film. The sternal marrow showed no parasites or malaria pigment. Pseudo-agglutination was marked and the sedimentation rate rapid. The plasma was dark brown in colour ; oxyhaemoglobin = 60·0 mg. per 100 c.c. ; pseudo-methaemoglobin (extinction coefficient = 3·5) and bilirubin = 33 units (indirect). Blood urea = 88 mg. per 100 c.c. The urine was black in colour ; oxyhaemoglobin = 168·3 mg. per 100 c.c. ; methaemoglobin = 226·25 mg. per 100 c.c. ; much urobilin. Though leucocytes were plentiful, granular casts were not observed.

13.12.36.—Patient was desperately ill and very jaundiced. Temperature = 98·6° F. Pulse = 114 per minute. A large purpuric patch was noted on the inside of the left knee. Bilious vomiting, dyspnoea and restlessness were marked, the patient tossing about in bed. Blood pressure—S/D = 102/68. From 6 p.m. yesterday until midnight some 550 c.c. of dark black urine containing much oxyhaemoglobin and urobilin were passed. Later retention was suspected ; a catheter was passed at 11 a.m. and 450 c.c. of brown urine were obtained.

R.B.C.'s = 780,000 per c.mm. Pseudo-agglutination marked ; sedimentation rate very rapid. Haematocrit = 9·8. Reticulocytes were scanty and no malarial parasites were found. The plasma was dark brown in colour and contained a large amount of pseudo-methaemoglobin, (extinction coefficient = 5), but only a small quantity of oxyhaemoglobin, *i.e.* 23·1 mg. per 100 c.c. Bilirubin = 38 units (indirect). Blood urea = 143 mg. per 100 c.c. The urine (11 a.m. specimen) contained no methaemoglobin, only a trace of oxyhaemoglobin (15·8 per 100 c.c.), much urobilin, numerous epithelial cells and a few leucocytes, red cells and granular casts.

The patient died at 8.30 a.m., some 80½ hours after onset of haemoglobinuria. No autopsy was possible, but a quantity of clear urine was obtained by catheterization after death.

COMMENT.—The cause of death was anaemia. Kidney function, as indicated by the excellent water secretion (polyuria), was satisfactory throughout the illness, and multiple blood transfusions would undoubtedly have saved the patient. Intravascular haemolysis probably ceased some hours before death and the urine obtained at postmortem was clear. The plasma findings indicated a progressive increase in the amount of pseudo-methaemoglobin in the plasma at the expense of the oxyhaemoglobin. Oxyhaemoglobin had practically disappeared from the plasma on the day of death, whereas pseudo-methaemoglobin was present in very large amounts. Despite this fact the latter pigment was never demonstrable in the urine.

CASE XIX.

A girl, aged 6 years, had suffered from malaria for 3 years, but never previously from blackwater. She had a rigor on the previous evening and next morning (13.12.36), about 6.30 a.m. took 3 grains of quinine sulphate. At 1 p.m., *i.e.*, 6½ hours later, she passed black water.

14.12.36.—The child was examined in her two-roomed home in the village of Nea Halkithon about 5 p.m. There was marked anaemia, yellow tinting of the conjunctivae and skin and tender enlargement of the spleen which extended to the level of the umbilicus (++).

Laboratory Investigations.—Parasites (M.T.) were found in thick blood films by Dr. A. KONDI. Pseudo-aggluntination was present and the sedimentation rate was rapid. Haematocrit = 22·9. Reticulocytes were scanty. The plasma was dark brown in colour and contained oxyhaemoglobin = 55·4 mg. per 100 c.c., pseudo-methaemoglobin (extinction coefficient = 3), and bilirubin = 19 units (indirect). Blood urea = 43 mg. per 100 c.c. The urine (2 p.m. specimen) was dark red, acid in reaction and contained oxyhaemoglobin = 157·8 mg. per 100 c.c., methaemoglobin = 271·5 mg. per 100 c.c., much urobilin, but no pseudo-methaemoglobin. There were a few leucocytes, epithelial cells and granular casts.

15.12.36.—The child was very anaemic and was still passing dark red urine of acid reaction. It contained oxyhaemoglobin (205 mg. per 100 c.c.), methaemoglobin (271·5 mg. per 100 c.c.), much urobilin and a few granular casts and leucocytes. By 6 p.m. the urine had cleared. She was treated with abundant fluids, alkalis, and atebrin, and subsequently made an uninterrupted recovery.

COMMENT.—This was the most severe of the three cases observed amongst children in our series; she was the only one whose plasma showed pseudo-methaemoglobin, the haemolysis in the others being milder and of less than 24 hours' duration.

CASE XXII.

Female, aged 38 years, had chronic malaria all the summer. Feeling unwell, she had 2 tablets (6 grains) of quinine sulphate before going to bed. Next morning (13.12.36) 6 grains were again taken at 6.30 a.m. At 1 p.m., she developed a rigor and fever and passed black water. Bilious vomiting ensued next day and the haemoglobinuria continued.

15.12.36.—She was admitted to the Refugee Hospital, Salonika, at 8 p.m., some 55 hours after onset. Temperature = 103·4° F.

16.12.36.—Examination next morning about 11 a.m. revealed a very restless and jaundiced patient. Temperature = 100·8° F. Pulse = 120. Respirations = 20 per minute. Bilious vomiting was troublesome. The tongue was furred, but still moist, the spleen was enlarged to the level of the umbilicus (++) and there were petechial haemorrhages into the skin of the abdomen and arms. Blood pressure—S/D = 140/68. For the first 12 hours after admission 150 c.c. of blackish urine were passed.

Laboratory Investigations.—R.B.C.'s = 2,375,000 per c.mm. Reticulocytes = 0·1 per cent. Blood group II (Moss). Haematocrit = 19·07. Mean corpuscular volume = 80·3 c. microns. No parasites in blood smears. The plasma was dark red in colour and contained oxyhaemoglobin (522·1 mg. per 100 c.c. or 3·73 per cent.), pseudo-methaemoglobin (extinction coefficient = 3), and bilirubin = 11 units (indirect). Blood urea = 155 mg. per 100 c.c. The urine was redish-black in colour, neutral to litmus and contained oxyhaemoglobin (89·4 mg. per 100 c.c.), methaemoglobin (454 mg. per 100 c.c.) and urobilin. Casts and leucocytes were not demonstrable. Urine passed in 24 hours = 1,600 c.c.

Treatment.—This consisted of daily intramuscular injections of isotonic glucose solution (500 to 750 c.c. per day) and intravenous injections of calcium chloride. Camphor, caffein, digitalin and euphyllin were also given, and in addition 200 c.c. of hypertonic glucose solution when anuria appeared imminent.

17.12.36.—Patient's condition worse, vomiting much brownish-green material. Blood pressure—S/D = 104/68. Tongue coated, but still moist. The abdominal muscles were very tender over the liver and spleen which was palpable below the level of the umbilicus. Some rhonchi noted in the right lung

R.B.C.'s = 1,585,000 per c.mm. The plasma was dark red and contained oxyhaemoglobin = 346·5 mg. per 100 c.c., pseudo-methaemoglobin (extinction coefficient = 4)

and bilirubin = 11 units (indirect). Blood urea = 253 mg. per 100 c.c. The urine was acid in reaction and contained oxyhaemoglobin (100 mg. per 100 c.c.) and methaemoglobin (316·75 mg. per 100 c.c.). Urobilin was present. No casts, but a few leucocytes and a large number of epithelial cells were found. Quantity of urine passed up to mid-day was 850 c.c.; none later.

18.12.36.—The patient was very jaundiced. The tongue was brown and dry while the veins contained little circulating blood. She had not micturated since noon on 17.12.36, so at 11 a.m. a catheter was passed. Only 10 c.c. of brown urine were obtained; this was acid in reaction and contained oxyhaemoglobin (23·6 mg. per 100 c.c.), and methaemoglobin (90·5 mg. per 100 c.c.). Urobilin, granular casts, leucocytes and epithelial cells were present. The plasma was dark red and contained oxyhaemoglobin (92·4 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 5·5) and bilirubin = 10 units (indirect). Blood urea = 187 mg. per 100 c.c. By 4.30 p.m. the patient was visibly shrinking from dehydration. Though dyspnoeic she lay uncannily still in bed.

19.12.36.—Died at 7 a.m. in a collapsed condition with subnormal temperature = 97·1° F.

Autopsy.—The liver and spleen were enlarged and congested, and the gall bladder filled with dark blackish bile. The kidneys were swollen and the vessels engorged and congested; the cut surface appeared darkish red on section.

Microscopic Pathology. Liver.—There was cloudy swelling and some loss of cellular outline and pattern. Much haemosiderin was present, but the Küpffer cells were almost entirely free of malaria pigment.

Spleen.—The capsule was markedly thickened and the organ very congested and cellular; the Malpighian corpuscles were inconspicuous. Malarial pigment was not evident.

Kidneys.—The glomeruli were normal, but the tubules showed a tremendous amount of damage. Toxic changes and desquamation were evident in the tubular epithelium of the cortex where some blockage occurred, while in the medulla the majority of collecting tubules were completely filled up with eosinophil-staining, homogenous or granular debris, their appearance contrasting with the adjacent vessels which were congested and filled with red cells.

COMMENT.—This patient died from anuria, uraemia and renal acidosis on the 7th day of the disease. The pathological basis for this was the large proportion of the tubules which were clogged with eosinophil debris deposited from excreted blood pigment; degeneration of the tubular epithelium was also a contributory factor.

Investigation of the plasma for circulating pigments showed the highest concentration of oxyhaemoglobin which we have yet observed, *i.e.*, 522 mg. per 100 c.c. or 3·73 per cent. on the 4th day of the disease. Pseudo-methaemoglobin was also present; the extinction coefficient = 3. Subsequently the oxyhaemoglobin decreased, equalling 346·5 mg. per 100 c.c. or 2·475 per cent. on the 5th day, and 92·4 mg. per 100 c.c. on the 6th day, whereas the extinction coefficient of pseudo-methaemoglobin steadily increased, from 4 on the 5th day to 5·5 on the 6th day of the illness. The sum total of circulating extracorpuscular blood pigments (oxyhaemoglobin plus pseudo-methaemoglobin) must have been very considerable in this case.

CASE XXIII.

M., aged 21 years, a refugee from Bulgaria living in Macedonia, was subject to chronic malaria which was particularly troublesome that summer. He was in the habit of taking quinine sulphate (12 grains) when he had fever and also at other times when he remembered to do so.

16.12.36.—He developed fever, took 6 grains of quinine at 6 a.m. and 6 grains at 9 a.m. At 11 a.m. he had rigor and half-an-hour later passed black water. The present was his first attack of blackwater; his sister had suffered from it 10 years ago.

17.12.36.—The patient was admitted to hospital. Temperature = 100·8° F. Icteric tinge in skin and conjunctivae. Enlargement of the spleen which mainly extended to the umbilicus. Blood pressure—S/D = 108/65. One malarial parasite (ring form) was found in smears of sternal marrow. Haematocrit = 27·25. The plasma was dark red in colour and contained oxyhaemoglobin 124·7 mg. per 100 c.c. and pseudo-methaemoglobin (extinction coefficient = 2). Bilirubin = 10 units (indirect). Blood urea = 86 mg. per 100 c.c. Pseudo-agglutination marked and sedimentation rate rapid. The urine was dark red, acid in reaction and contained urobilin, oxyhaemoglobin (284 mg. per 100 c.c.) and methaemoglobin (181 mg. per 100 c.c.). No casts but a few leucocytes and numerous epithelial cells were noted.

Treatment.—For 5 days 1,000 c.c. of isotonic glucose were injected intramuscularly and calcium chloride intravenously. Stimulants including camphor were administered.

18.12.36.—Temperature = 99° F. Patient more jaundiced. Passed 700 c.c. of dark red urine from 12 noon until 8 a.m to-day. R.B.C.'s = 2,600,000 per c.mm. Haematocrit = 19·6. Mean corpuscular volume = 75·4 c. microns. Plasma still dark red, containing oxyhaemoglobin (83·2 mg. per 100 c.c.) and pseudo-methaemoglobin (extinction coefficient = 3). Bilirubin = 16 units (indirect). Blood urea = 82 mg. per 100 c.c. The urine was dark red in colour, neutral to litmus and contained oxyhaemoglobin (278·8 mg. per 100 c.c.), and methaemoglobin (362 mg. per 100 c.c.). Urobilin present. Some granular casts in addition to leucocytes and epithelial cells were now noted.

19.12.36.—Patient rather weak and hysterical. Bilious vomiting troublesome throughout the night. Passed 800 c.c. of urine; 6 a.m. specimen contained no blood pigments. Plasma was light golden-brown in colour. Only a trace of oxyhaemoglobin (23·1 mg. per 100 c.c.), but there was still a quantity of pseudo-methaemoglobin (extinction coefficient = 2·5). Bilirubin = 5·5 units (indirect). Blood urea = 168 mg. per 100 c.c. The urine was acid to litmus and contained no oxyhaemoglobin or methaemoglobin, but considerable amounts of urobilin. Granular and epithelial casts undergoing granular degeneration were plentiful as well as epithelial cells and some leucocytes. Quantity = 1,100 c.c.

20.12.36.—Patient looks more anaemic since jaundice has disappeared. R.B.C.'s = 1,895,000 per c.mm. Haematocrit = 13·6. Mean corpuscular volume = 71·76 c. microns. Reticulocytes = 0·1 per cent. The plasma was light brown and contained only 1·5 units of bilirubin (indirect). Blood urea = 138 mg. per 100 c.c. The urine was pale, acid to litmus, and contained a trace of urobilin and a few casts, red cells, leucocytes and many uric acid crystals. Quantity = 1,900 c.c.

21.12.36.—Patient improving. Urine clear: quantity = 1,000 c.c. R.B.C.'s = 1,815,000 per c.mm. Reticulocytes = 3·4 per cent. Plasma bilirubin = 0·5 units (indirect). Blood urea = 81 mg. per 100 c.c.

24.12.36.—Still improving. Urine clear: quantity = 1,050 c.c. Reticulocytes = 1·7.

25.12.36.—Urine clear: quantity = 1,600 c.c. Reticulocytes = 8·1 per cent. Subsequent progress satisfactory. Discharged within 3 weeks.

COMMENT.—The case was one of moderate severity, the haemoglobinuria lasting approximately $2\frac{1}{2}$ days. Oxyhaemoglobin reached its maximum concentration (124·7 mg. per 100 c.c.) on the 2nd day and pseudo-methaemoglobin on the 3rd day, while it had only slightly decreased on the 4th day at a time when there was only a trace of oxyhaemoglobin in the plasma and the urine had cleared. A maximal reticulocytosis of 18·7 per cent. occurred 9 days from onset.

Case XXIV.

Male, aged 34 years, lived in Salonika. A brother had died of blackwater fever. In June and July, 1936, the patient went to an adjacent village, where he contracted malaria. As a rule, he took 6 grains of quinine sulphate daily, but on 16.12.36 he had increased this to 18 grains as he wanted to prevent a relapse. On 17.12.36, at 7 a.m., he had 9 grains and at noon he passed black water; at 1 p.m. he had a rigor. He was admitted to hospital at 8 p.m., and was given 500 c.c. of glucose intramuscularly. Temperature = 102·6° F.

18.12.36.—Temperature = 102·2° F. Pulse = 94 per minute. Perspiring profusely. Tongue furred, but moist. Upper abdominal muscles held too rigidly to palpate either spleen or liver. Slight jaundice. R.B.C.'s = 4,270,000 per c.mm. No parasites. Haematocrit = 37·0. Mean corpuscular volume = 88·1. Blood group II (Moss).

The plasma was dark red in colour and contained oxyhaemoglobin (170·9 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 1·5) and bilirubin (16 units). The urine, which was slightly acid in reaction, was dark red in colour and contained oxyhaemoglobin (189·4 mg. per 100 c.c.), methaemoglobin (364·5 mg. per 100 c.c.), urobilin, casts and a few leucocytes and epithelial cells. Blood urea = 56 mg. per 100 c.c.

Atebrin, 0·1 gramme, was injected and 500 c.c. of isotonic glucose given intramuscularly.

19.12.36.—Temperature = 101·2° F. Haemoglobinuria still present. Jaundice marked, hiccough troublesome and spleen tender and palpable well below the costal margin. The plasma was golden brown in colour and contained oxyhaemoglobin (60 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 2) and bilirubin (11 units). Blood urea = 99 mg. per 100 c.c. Polyuria was present. Two litres of slightly alkaline urine were passed in last 24 hours, containing oxyhaemoglobin (142 mg. per 100 c.c.), urobilin and a few casts. R.B.C.'s—3,150,000 per c.mm. Haematocrit—25·6. Corpuscular volume—81·3 c. microns. Reticulocytes were less than 0·1 per cent.

20.12.36.—Temperature = 100·2° F. Pallor was increased. Hiccough less troublesome. The plasma was light brown in colour and contained oxyhaemoglobin (23·1 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 1·5) and bilirubin (2 units). Blood urea = 81 mg. per 100 c.c. The urine, which was alkaline in reaction, was light brown in colour and contained bilirubin, but no blood pigment and very few casts. R.B.C.'s = 2,610,000 per c.mm. Haematocrit = 20·16. Mean corpuscular volume = 77·0 c. microns.

The subsequent progress of the patient was satisfactory.

21.12.36.—Temperature = 100·2° F. The plasma was pale yellow in colour and contained 1·75 units of bilirubin, but no oxyhaemoglobin or pseudo-methaemoglobin. Blood urea = 51 mg. per 100 c.c.

22.12.36.—Temperature = 101·2° F. Urine clear. R.B.C.'s = 2,375,000 per c.mm. Reticulocytes = 3·6 per cent.

25.12.36.—R.B.C.'s = 2,230,000 per c.mm. Reticulocytes = 13·9 per cent.

Comment.—This patient in the initial stages looked a sick man, but recovered despite severe hiccough which clinically is generally-regarded as of grave prognostic significance.

The haemoglobinuria lasted approximately 2½ days. Water excretion, as indicated by polyuria, was satisfactory and the blood urea never exceeded 99 mg. per 100 c.c. The kidneys, therefore, were at no time seriously implicated.

The plasma oxyhaemoglobin on the 2nd day equalled 170·9 mg. per 100 c.c. It decreased on the 3rd day to 60·0 mg. per 100 c.c. and was barely demonstrable in significant quantity on the 4th day of the disease. Pseudo-methaemoglobin, on the other hand, reached its greatest concentration on the 3rd day (extinction coefficient = 2), and was present in appreciable quantity on the 4th day (extinction coefficient = 1·5) after oxyhaemoglobin had practically disappeared.

The reticulocytes on the 9th day equalled 13·9 per cent. and the subsequent haematological response was satisfactory.

CASE XXVII.

Male, aged 16 years, lived in Salonika, but had been to an adjacent village for the summer. There was no family history of blackwater. He developed malarial fever 15 days earlier, for which he took quinine sulphate. On 19.12.36, he was admitted to hospital with splenomegaly and received plasmoquine, 0·03 gramme daily, as well as quinine, 1·5 to 2 grammes daily, for 4 days. At about 6 p.m. on 23.12.36, he started vomiting; a rise in temperature to 101° F. followed, but it was not until 5 a.m. next morning that the urine was noted to be black.

24.12.36.—Temperature = 104·4° F. Anaemia and a slight icterus noted. The spleen was enlarged. The vomitus was a spinach green. Atebrin (0·1 gramme) was injected and 500 c.c. of isotonic glucose and 250 c.c. of physiological saline were given intramuscularly.

Laboratory Investigations.—R.B.C.'s = 2,355,000 per c.mm. Pseudo-agglutination and a rapid sedimentation rate were present. Reticulocytes = 13·7 per cent. Haematocrit = 18·0. Corpuscular volume = 76·3 c. microns. The plasma was a dark reddish brown colour and contained oxyhaemoglobin (55·4 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 3·5) and 5·0 units of bilirubin (indirect). Blood urea = 80 mg. per 100 c.c.

The urine was dark brown in colour, neutral to litmus and contained oxyhaemoglobin (42·1 mg. per 100 c.c.) and urobilin. There were a few granular and epithelial casts and numerous epithelial cells.

25.12.36.—Fever subsided and the urine cleared at midnight. R.B.C.'s = 2,240,000 per c.mm. Reticulocytes = 14·3 per cent. Haematocrit = 16·9. Mean corpuscular volume = 75·4 c. microns. The plasma was yellowish-brown in colour; it contained no significant quantity of oxyhaemoglobin, though pseudo-methaemoglobin was still present (extinction coefficient = 1·5). Bilirubin = 2·75 units (indirect). Blood urea = 55 mg. per 100 c.c. The urine was clear, acid to litmus and contained a small quantity of urobilin, but no blood pigments. There were a few granular casts, epithelial cells and leucocytes. A course of atebrin (0·3 gramme daily for 5 days) followed by plasmoquine (0·01 gramme daily for 6 days) was given. Subsequently he received two intramuscular injections of quinine (15 and 22 grains) and oral quinine (18 grains daily) for 1 week. Haemoglobinuria did not recur, but there were short bouts of fever.

12.1.37.—The temperature was normal, but the patient was still pale and weak. The van den Bergh reaction was 1·4 units (indirect).

COMMENT.—This case developed haemoglobinuria in hospital; its duration probably did not exceed 30 hours. Both oxyhaemoglobin and pseudo-methaemoglobin were present in the first sample of plasma, but only pseudo-methaemoglobin was found next day. The high reticulocyte counts of 13·7 per cent. and 14·3 per cent. at this early stage are attributable not to the anoxaemia associated with the haemolysis of blackwater fever, but to the effect of anti-malarial treatment in a patient suffering from malaria anaemia.

CASE XXVIII.

Female, aged 28 years, was a refugee from Pontos and stated that her mother had blackwater fever some years ago. She came from a village which had a bad reputation for blackwater fever. She was subject to a chronic malaria and took oral quinine frequently. On 23.12.36, she received an injection of 7·5 grains of quinine bihydrochloride at 4 p.m. At 9 p.m. she had a rigor and passed black water.

24.12.36.—At 6 p.m. there was another rigor. Urine passed at 8 p.m. was darkish red in colour and contained oxyhaemoglobin (121·0 mg. per 100 c.c.) and methaemoglobin (905 mg. per 100 c.c.). Urobilin was plentiful. No further urine was obtained naturally and anuria set in.

26.12.36.—When the patient was first seen she had grunting respirations, was markedly anaemic and jaundiced and had an enlarged spleen extending to the umbilicus. Blood

pressure—SD = 108/65. The plasma was golden brown in colour and contained oxyhaemoglobin (37·0 mg. per 100 c.c.), pseudo-methaemoglobin (extinction coefficient = 3) and bilirubin (5 units). Blood urea = 244 mg. per 100 c.c.

A catheter was passed at 8 a.m., and again at 3 p.m., and on the last occasion a few c.c. of dark red urine were obtained. It was acid in reaction and contained 21·0 mg. per 100 c.c. of oxyhaemoglobin and 226 mg. per 100 c.c. of methaemoglobin. Much urobilin and a few granular casts were also present.

COMMENT.—We were subsequently informed that the patient died with anuria and malaria on 1.1.37, some 10 days from the onset of haemoglobinuria.

APPENDIX II.

ANAEMIA.

The other aspect of the investigation concerns the haematological findings which will form the subject of a subsequent paper. In the meantime, passing reference to it might not be out of place.

Our most vivid clinical impression of the hospital population in Salonika and that of adjacent Macedonian villages was the frequency and severity of the anaemia encountered there. Similarly, the outstanding differences in Macedonian patients with blackwater fever compared with those of our London series was the persistance of anaemia due to sluggish blood regeneration.

Atypical clinical syndromes in the post-haemoglobinuric phase were unduly frequent and it was only near the end of the investigations that we realised such patients presented a megalocytic blood picture.

A number of pregnant women with splenomegaly and severe anaemia were in the wards of the Refugee Hospital at Salonika. The first case investigated from this viewpoint proved to be an example of tropical macrocytic anaemia.

After returning to London, and working out the Price-Jones curves and bone marrow findings in material collected from cases of malaria and blackwater fever, the widespread distribution of this nutritional deficiency became even more apparent.

In order to understand conditions of life in Macedonia it is necessary to remember that over 700,000 refugees from Asia Minor and elsewhere have immigrated there during the past 15 years and been settled in villages scattered through different parts of the country, many of which are highly malarious. Economically, conditions are hard, poverty is rife, diet and nutrition defective. Mr. H. FOY and Dr. KONDI inform us that white flour is widely used for bread-making and that red meat and eggs are rarely consumed. The rice in common usage is small-grained and well milled and all the available data indicate that there is a deplorable deficiency in foods containing extrinsic factor.

Our observations on this side-line are naturally incomplete, but as tropical macrocytic anaemia has never previously been recognized in Salonika and as the disease is curable by marmite and preventible by correct dietary, the problem exceeds in practical importance even that of blackwater fever and urgently requires attention.

Discussion.

The President (Col. S. P. James) : We have heard these two most interesting papers and I now propose to ask you to discuss them. Before I do so I would like to say that before I left Cambridge this morning I had a word or two with Professor Keilin on the subject of Dr. Hamilton Fairley's paper, and he expressed his great regret that he could not be here to-night to speak on it himself. He considers that the discovery described is exceedingly interesting and important, and he said that if on any future occasion we should discuss the subject of haemoglobin in our Society he would be most pleased to be invited to attend. He thought it would be interesting if Dr. Fairley would record the percentage of laked blood which is converted into pseudo-methaemoglobin and if he would give further particulars of conditions other than blackwater fever in which this new pigment is formed. The papers are now open for discussion.

Sir Rickard Christophers : Those who have investigated blackwater fever have always been met by two great difficulties : the first is the difficulty of getting cases, the second is, having got the cases, to get anything new about blackwater fever out of them. The paper by Mr. Foy and Dr. Kondi seems to me, apart from anything else, to have a very important bearing upon the first of these difficulties. In the tropics blackwater fever usually occurs amongst a scattered European population living under very primitive conditions where the means of communication are difficult. The result is that cases really suitable for investigation are hard to obtain. At a time when people in what was then British Central Africa were much concerned about the prevalence of blackwater fever, Professor Stephens and myself were able to get only some five or six cases in as many months; and when with Dr. Bentley, in Assam, we were about 18 months in getting together some twenty-three cases which we were able to investigate at all fully. To hear Mr. Foy speaking of 400 cases is something quite extraordinary in this disease. One had heard of very large numbers of cases described by the Greek physicians in that country, but one could not help wondering whether these could really be the same disease as blackwater fever in the tropics. Mr. Foy to-night has quite clearly settled this point. This great prevalence of blackwater fever in Greece is an extremely important fact about the disease. Mr. Foy's figures are very interesting, especially his table giving the monthly number of malaria and blackwater fever cases. I am afraid it would take too long to discuss these at all adequately. There is one question I would like to ask Mr. Foy in reference to the proof of his paper, *viz.*, whether in Table II, where he gives a column designated " Percentage of Malaria that are Blackwater Fever," this column is not the relation of total admissions of malaria to admissions for blackwater fever. I presume this is so. I ask because it would be very interesting to know what was the proportion of blackwater fever cases occurring in patients admitted for malaria as against

that in patients admitted for other conditions. Possibly such figures are not available, but if they were they might give confirmation to the view that blackwater fever is dependent on previous malarial infection.

Dr. FAIRLEY and Mr. BROMFIELD'S paper records quite new facts about the disease. These authors for some years now have been working systematically in the collection of biochemical data regarding blackwater fever. I do not think I shall be far wrong in saying that for the last 20 years theirs has been almost the only work adding anything really new to what is known of the essential character of this disease. Nevertheless, in spite of its interest and importance in understanding some features in the disease the new pigment they have described is like any other product of red cell destruction in blackwater fever. It shows evidence of destruction of the red cells, but does not directly touch the fundamental question of the real cause of blackwater, apart that is from the fact now almost universally recognised that it is the result of long, or comparatively long, pre-existing malaria.

It is perhaps not uncommon to think of blackwater as a very interesting disease, but not really one of major importance. This, in my opinion, is wrong. Probably to the European nations blackwater fever is economically the most important of all tropical diseases. It is this disease which has given Africa the name of " The White Man's Grave." It is the disease of which people in many parts of the world are most afraid, that prevents the colonization of new areas, and prevents the success of many commercial and other projects. There is therefore all the greater need that we should really try to arrive at the causation of this condition.

Professor Warrington Yorke: I have always been extremely interested in blackwater fever, partly, I suppose, because nobody who is concerned with the study of tropical medicine can fail to be impressed with its dramatic onset and course, and partly because it was with the object of endeavouring to find out something of the mechanism of the disease that I first went to Central Africa with Dr. BARRATT 30 years ago. Unfortunately, of later years other interests and other duties have prevented my working on the disease; but I have turned to it from time to time in a dilatory sort of way, and it has fallen to my lot to review for the *Tropical Diseases Bulletin* all the papers published on the subject during the last 25 years. When one goes through that lengthy list one cannot fail to be impressed with the number of them that relate to the treatment of the disease. The various writers advocate some particular measure of their own, for which they claim outstanding success. But a few years ago I took the trouble to go through the figures relating to blackwater fever in one of our African dependencies—Southern Rhodesia, I think—from 1914 to 1928, and I found that although the fatality rate varied greatly from year to year it showed no general tendency to decline during the 15 years in question. The ordinary simple person, like myself, can only conclude from this that the treatment of the disease

has not improved very much during that period. But how could it improve? We have learned practically nothing of the disease during the last 25 or 30 years.

I am able to go back, I think, to the state of knowledge, or ignorance, that existed in 1907, and I remember that the view, which found most favour, of the mechanism of the most important sign, haemoglobinuria, was that of PLEHN, who said that the haemoglobin in the urine came from haemorrhage into the kidneys, and that the red cells were destroyed in the tubules of the kidneys. There are still people who support that view, but it does not receive general favour. I remember that Dr. BARRATT and I insisted that haemoglobinuria was consequential and dependent on haemoglobinaemia; and CHRISTOPHERS and BENTLEY, about the same time, pointed out the same fact. We insisted that if knowledge on the mechanism of this disease was to advance we must introduce quantitative methods into our examinations. I think Dr. HAMILTON FAIRLEY and later workers have fully confirmed that view, and Dr. FAIRLEY has gone further because he has explained a phenomenon which was very puzzling to me, and, I think, to all the early workers, and that is, Why is the oxyhaemoglobin in the plasma so small in amount? He has explained to-night why that is the case. I have the greatest admiration for the work of Dr. Fairley and Mr. Foy on this disease, but there is still so much to be done. I think there is no doubt at all that in blackwater fever the haemolysis is due to a sudden and catastrophic crisis, or to a series of such crises, somewhere in the circulation; but, as Sir RICKARD CHRISTOPHERS has pointed out, nobody knows what precipitates this crisis, and that, after all, is the essence of the whole thing; and nobody knows where the crisis occurs, whether in the general circulation or in some backwater of the circulation.

There are one or two other points to which I would like to refer. Why is it that in diseases like *Babesia* infection, for example, and in paroxysmal haemoglobinuria, there is so much free oxyhaemoglobin in the plasma, whilst in blackwater fever, where the haemolysis is at least as great, in many cases far greater, you can find very little oxyhaemoglobin? I wonder whether the relative deficiency of oxyhaemoglobinaemia in blackwater fever is bound up in some way with a proliferation of the reticulo-endothelium due to antecedent malaria: I do not know, and I do not think anybody else knows. It is common knowledge, of course, that the main cause of death in this disease is suppression of urine and uraemia. As Dr. FAIRLEY has pointed out, NAUSS and I investigated this question experimentally in 1911. We reached certain conclusions, and they have been confirmed by Dr. FAIRLEY and others. It is a curious fact that only 10 per cent. at the most—sometimes much less—of the destroyed blood is eliminated through the kidneys. Dr. FAIRLEY has offered an explanation of that, and possibly he is correct. It is interesting to note that the same fact—that only 10 per cent. is eliminated from the kidneys—is seen when you introduce a solution of its own haemoglobin into the circulation of the rabbit. In paroxysmal haemoglobinuria not more than 10 per cent. of the free haemoglobin

in the plasma gets out through the kidneys, and the same applies to *Babesia* infections. The manner of the escape of the haemoglobin from the plasma into the urine is, I think, of the greatest importance. NAUSS and I produced what, to our minds, was very weighty evidence that haemoglobin is actually secreted into the urine through the epithelium of the convoluted tubules, and that this fact played a considerable rôle in the degenerative changes leading up to suppression of urine. I should be interested to hear Dr. FAIRLEY's view on the matter. I still believe that it is this overwork of the renal epithelium that is partly responsible for the degeneration which occurs and which aids in the suppression of urine.

Sir RICKARD CHRISTOPHERS has drawn attention to what has always been one of the greatest difficulties in investigating this disease, namely, that of getting together a sufficient number of cases in a sufficiently short time and within a small enough area. Sir RICKARD CHRISTOPHERS has told us of the number of cases he encountered when he was in Nyasaland. When BARRATT and I were there a few years after CHRISTOPHERS we saw, in 18 to 20 months, fewer cases than FAIRLEY has seen in Macedonia in 6 short weeks. I quite agree with all that Sir RICKARD CHRISTOPHERS said about the importance of this disease to the European in the tropics. It is certainly one of the more important tropical diseases, not merely because of its death-rate, which is considerable, but also because of the demoralization which it produces in the patient, and of the dread of it induced in all people who suffer from frequent attacks of malaria. The problems which the investigation of blackwater fever presents are certainly complicated ; but if they were properly attacked by an adequate team of workers there is no reason, in my judgment, why they should not be solved. I hope, therefore, that every encouragement will be given to Dr. FAIRLEY and Mr. FOY to prosecute their studies in this part of the world, which is so peculiarly adapted for that purpose.

Dr. Gilles de Kock (Onderstepoort, South Africa) : I am very pleased to have been present to listen to the papers and the discussion to-night. I would like to bring to your notice observations which have been made at the Onderstepoort Laboratory, South Africa, in respect of a disease in sheep characterized by haemoglobinaemia and methaemoglobinaemia. We have carried out haematological as well as chemical and pathological studies. The aetiology of the disease is still obscure. So far, we are not able to account for the sudden and tremendous destruction of red cells in the circulation, accompanied by an excretion of their broken down pigments into the urine. Spectroscopically we also identified methaemoglobin, but in view of Dr. FAIRLEY's investigations, this whole matter will be reopened. The sudden extensive haemolysis seems to be associated with a change of environment and at the present moment our views are that the condition may be associated with some nutritional or dietetic factor. The pathology of the liver reveals very interesting pigment cells and we are of the

opinion that these cells are probably not reticulo-endothelial cells, but so-called reticulum cells (?). This pigment is not found in the sinus cells, but in the cells of the lymph follicle of the lymph glands. In conjunction with Dr. REMINGTON, micro-chemical tests have been carried out to identify the nature of the pigment in these cells. It is insoluble in absolute alcohol and stains intensely with sudan III. In the liver these pigment cells are associated with an interstitial hepatitis and their presence in this position makes it possible for us to arrive at a diagnosis. The literature of blackwater fever has been carefully scrutinized to ascertain whether anything of this nature has been described.

This haemoglobinaemia and methaemoglobinaemia in the sheep seems to be associated with an increase of copper content in the liver substance. According to preliminary investigations, the clinical healthy sheep shows about 10 mgm. per 100 grammes of liver substance, whereas in this disease this amount has been greatly exceeded. What is the significance of the copper in this breaking down process of haemoglobin and in what relation does it stand to copper poisoning which seems to produce more or less a similar pathological picture ? In the field we were, however, not able to attribute this enzootic methaemoglobinaemia to copper poisoning.

The deliberations and activities of your Society are being very closely followed by us and your reports are always read with a tremendous amount of interest.

Dr. C. C. Chesterman : May I ask Mr. FOY three questions ? First, does he consider infection with quartan malaria gives some degree of immunity from blackwater fever ? Secondly, are we to understand that he considers that infection with *Plasmodium vivax* has some causal relationship to blackwater fever ? In view of the fact that parasites often disappear during blackwater fever (as he has noted himself) and that *P. falciparum* probably disappears more readily than *P. vivax*, it makes one wonder whether he was not dealing with mixed infection. Thirdly, does he consider the alkaline treatment for blackwater fever is now quite defunct ?

Major J. S. K. Boyd : Dr. FOY said that *P. vivax* seemed to be more commonly associated with blackwater fever than *P. falciparum*. I have had considerable experience of malaria parasites in Macedonia and the more that experience accumulated the more I was impressed by the number of mixed cases ; in fact every case was a potential one of mixed infection. I am talking of the state of affairs among British troops during the War. Although one form of the parasite may be prevalent in the peripheral blood at the time when blackwater fever occurs, there can be little doubt that all the native population has been infected with both forms at one time or another. Therefore, if sensitization with the malignant parasite is held to play any part in the aetiology of blackwater fever, it may safely be assumed that this factor is universally present in these Macedonian cases.

Mr. H. Foy (in reply): In reply to Sir RICKARD CHRISTOPHERS, the heading mentioned by him refers to the ratio of blackwater fever to malaria (*i.e.* $\frac{B}{M}$) and not to the number of blackwater fever cases occurring in patients admitted for malaria.

Dr. CHESTERMAN asked if infection with *malariae* may give some degree of immunity to blackwater fever. This is difficult to answer. There are villages in Macedonia which we have had under observation for the last 6 years, where originally *falciparum* formed 100 per cent. of the positives (spleen rate 100 per cent.; parasite rate 50 per cent.) but which latterly have shown a remarkable increase in quartan infection (80 per cent. in one year were quartan) and during the whole period only three cases of blackwater fever occurred. In contrast we have another village of the same size in the Peloponessus where the spleen and blood rates were only about half that of our Macedonian village, and quartan much rarer, but in the same period twenty-three cases of blackwater fever occurred. I should add that in the former village the population was 100 per cent. refugee, whilst in the latter it was 100 per cent. indigenous. We might further add in this connection that our figures show that there appears to have been an increase in *malariae* during the last 5 years or so.

Yes, I think there is most probably some relationship between blackwater fever and *vivax*, but, as Major BOYD has pointed out, this does not exclude previous sensitization with *falciparum*.

I think it quite likely that the proportion of mixed infection may have been higher than that actually found by us, if it is assumed that *falciparum* disappears more readily from the peripheral blood than *vivax*. On this point we would like to add that so far as our experience goes *falciparum* strains in Macedonia do not appear to be more sensitive than *vivax* to quinine.

I think that the alkaline treatment of blackwater fever is by no means defunct, but Dr. FAIRLEY will deal more fully with that point in his reply.

Major BOYD'S question with regard to the relative frequency of *vivax* and *falciparum* in blackwater fever cases can only be answered satisfactorily in cases where the frequency distribution of the species of parasites in the general population is known. The greater proportion of our blackwater fever cases that had *falciparum* in their blood can be accounted for by the greater abundance of this species in the population at large. Mixed infections are doubtless a very important factor, and as Major BOYD has said it is not impossible that the population has at some time or other been infected with both *falciparum* and *vivax*, so that previous sensitization with *falciparum* cannot be ruled out in the Macedonia blackwater fever cases.

As regards other questions which have been asked the answers to these will be found in the complete paper to be published in the TRANSACTIONS.

Dr. Hamilton Fairley (in reply): I will attempt to deal briefly with some of the questions which have been asked. I agree with Sir RICKARD CHRISTOPHERS in regard to the aetiology of blackwater fever. Our paper to-night illuminates the mechanism at work after the haemolysis has started, but has nothing to do with the fundamental cause of the haemolysis; indeed, it completely eliminates the theory we put forward 2 years ago regarding its cause. When we know the cause of haemolysis in blackwater fever we shall know practically everything we want to know. I do not altogether share Prof. WARRINGTON YORKE'S scepticism about our inability to do anything towards saving the lives of patients with blackwater fever. The work we have done in Macedonia and in London shows clearly one type of case associated with polyuria and adequately functioning kidneys in which there is prolonged haemolysis, and in which repeated blood transfusion saves life. Anyone who goes into this question on a more extensive scale simultaneously collecting adequate clinical, haematological and biochemical data, will be adding a very important chapter to the treatment of this disease. We saw cases die in Macedonia which we felt certain, from our experience in London, could have been saved by repeated transfusions. They died of anaemia. Their kidneys were secreting well. It would be a pity if anything said by Professor YORKE to-night were to prevent any of you who are going out to the tropics transfusing such cases. I also think alkalization of the urine is rational therapy in blackwater fever. Our work on pseudo-methaemoglobinaemia supports the experimental work of BAKER and DODDS in this connection since it shows there is no methaemoglobin in the blood. If the urine is made alkaline sufficiently early, clinical observation suggests there is less likelihood of producing silting-up of the renal tubules which Professor YORKE originally found to be the cause of anuria, and for which he and others have rightly advocated excessive fluid intake to flush out the kidneys. But, in addition, BAKER and DODDS have shown experimentally that if a urine has a pH of 6·0 or less and a salinity exceeding 1 per cent., methaemoglobin is formed from oxyhaemoglobin in the tubules; and blockage, with what they consider to be acid haematin, is likely to occur. That the alkaline treatment has often failed in anuric cases is true, but is not this mainly because massive haemolysis has already occurred and the renal damage been done before the reaction of the urine has been modified by treatment? BAKER and DODDS advocated alkalization, not to overcome blockage but to prevent it, and for this reason citrates and sodium bicarbonate should be immediately given not only in blackwater fever but in any intravascular haemolysis where lysis is likely to be continued or recurrent. Furthermore, by increasing the alkaline reserve, these drugs help to combat renal acidosis which we have demonstrated by biochemical methods to supervene in severe cases of this disease. Perhaps, in the future, the synthetic chemist may supply us with a drug which accelerates the conversion of oxyhaemoglobin into pseudo-methaemoglobin, thus protecting the kidneys from the dangers associated with haemoglobinuria.

In regard to the question asked by Colonel JAMES, we have only been working on this new pigment for a relatively short time and are not yet in a position to record its incidence in other diseases. In enterogenous cyanosis and drug poisonings methaemoglobin is formed within the corpuscles and the plasma has little opportunity of exerting its action since haemolysis rarely, if ever, occurs. Such cases are really examples of methaemoglobin-cythaemia and the pigment formed within the corpuscle is true methaemoglobin.

In other conditions, however, where the corpuscle is lysed and oxyhaemoglobin is liberated into circulating blood, the formation of pseudo-methaemoglobin is to be anticipated. In incompatible transfusion, for example, we noted some years ago the presence of oxyhaemoglobin and a pigment which spectroscopically resembled methaemoglobin. I am convinced the pigment which we were then observing was pseudo-methaemoglobin, and that this pigment will be found if it is looked for.

There is much to be said in favour of Professor YORKE'S views that free oxyhaemoglobin is selectively secreted by the epithelium of the tubules rather than being filtered through the glomerulus as physiologists hold. In the first place, it would be interesting to know whether the size of the molecule of oxyhaemoglobin is of the order which really permits its passage through the glomerulus: not all physiologists appear in agreement as to what actually is its molecular weight; though it is generally regarded as about 68,000. Secondly, the presence of eosinophil-staining globules in the tubular epithelium similar to those observed in the lumina of the tubules and the toxic degeneration which these cells undergo, suggest that haemoglobin or some toxic bi-product is being secreted by the kidneys. Of course it might equally well result from absorption of blood pigment or some toxic katabolite from within the lumina of the tubules. This, however, would not explain why in acute malarial haemolytic anaemia of monkeys (*P. knowlesi*), where blood destruction is very great, toxic changes are not infrequently found in the renal tubular epithelium though there is no suggestion of tubular blockage and no haemoglobinuria. The subject is one requiring further investigation and we hope our own experimental observations may throw some light on the subject at a later date.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937

COMMUNICATIONS.

CLINICAL OBSERVATIONS ON KALA-AZAR IN THE FUNG PROVINCE OF THE SUDAN.

BY

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Sudan Medical Service.

The following observations are based on the detection and treatment by the author of over 300 cases of kala-azar occurring in the Fung Province between 1933 and 1936.

Topography and Climate.

The disease occurred in villages situated on the banks of the Blue Nile and its tributaries from its entrance into the Sudan from Abyssinia down to about 150 miles short of its confluence with the White Nile at Khartoum, *i.e.*, between latitude 11 N. and 14 N. approximately. The river flows through an open flat plain of thick loam falling only about 200 feet in 200 miles.

The climate is tropical, minimum temperature rarely below 60° F., and annual mean relative humidity 50 to 55 per cent. The rainy season commences in April with about 10 mm., reaches its acme of about 200 mm. in August, and tails off by November.

*My thanks are due to the Director, Sudan Medical Service for permission to publish this paper and to Sir ROBERT G. ARCHIBALD, *C.M.G.*, *D.S.O.*, M.D., for assistance rendered in carrying out these observations.

Inhabitants.

The area is thinly populated by 200,000 Arabs concentrated in villages thickly dotted along the northern part of the river, 100,000 negroids widely scattered in straggly villages in the southern part, and 30,000 West African colonists, " Fellata," occupying fairly isolated large villages also in the northern part of the Province. The habits and mode of life of all these three are very much alike ; all deriving a more or less precarious livelihood from keeping cattle, sheep and goats, or by cultivating small patches of cereals.

Sanitation is very primitive ; the ground adjacent to villages, universally soiled, is mercifully re-sterilized by the strong sun. Spitting is promiscuous ; nasal secretion discarded haphazardly by the fingers on to the ground or clothing. Personal cleanliness is a very variable factor. Bathing in the river is frequently indulged in, but the advisability of including intimate clothing in the wash is not generally appreciated, hence louse infestation is common.

Housing conditions vary. The thatched mud hut of the few better-class natives is fairly commodious and clean, but that of the poorer people is small, filthy, ill-ventilated and liable to considerable overcrowding. The occupants sleep on beds made of strips of hide or rope which are often infested with bugs. The nocturnal population of the hut is often augmented by goats, sheep, dogs, cats, poultry, etc. Donkeys, cattle and camels are tethered as close to the owners' hut as possible.

Food is liable to considerable contamination in preparation and consumption owing to the abounding filth and flies, and the prevalence of communal feeding from the same receptacle. Water is usually drawn in clay receptacles from the river but natives not immediately on the bank will readily share a muddy pool with their animals. Villages at some distance from the river depend on deep wells for their supply in the dry season.

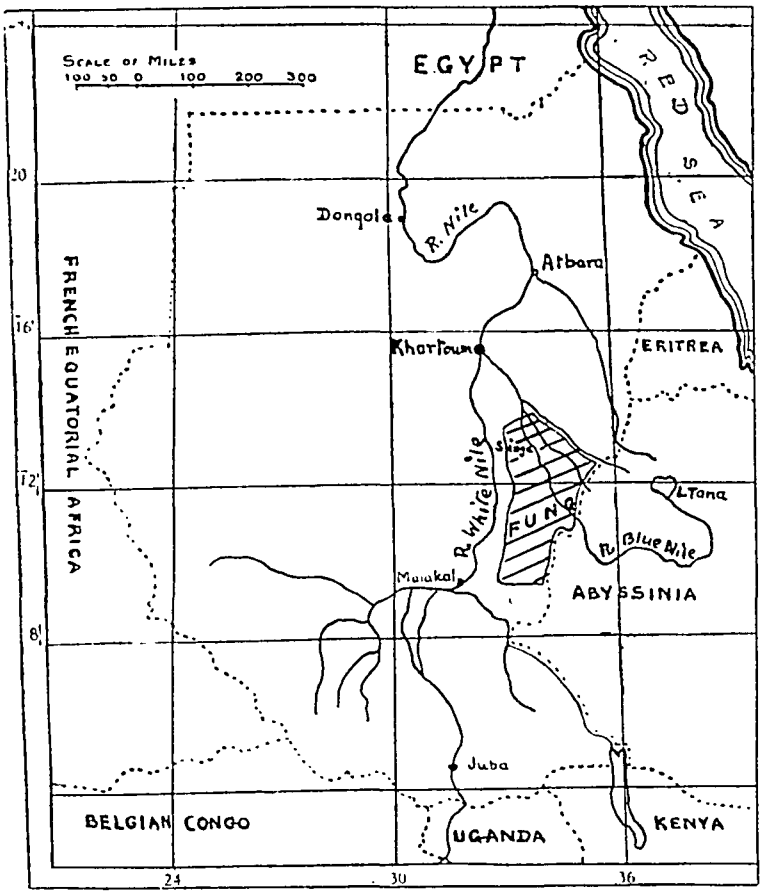
The staple article of diet is " kisra "—a kind of scone made from millet flour. A man will eat 3 or 4 lbs. of kisra a day, lubricating it with vegetable oil, " simsim," and washing it down with water, soup or milk. Vegetables are used sparingly. Meat consumption depends on personal affluence but in the poorer class rarely exceeds twice weekly. This diet though sufficient in calorie value (probably 3,000 to 4,000 calories) is obviously unbalanced, the carbohydrate being out of all proportion to the protein and fat content. The vitamin content is extremely variable. The occurrence of cases of scurvy and the low resistance to disease in general amongst the population suggests an inadequate supply of water-soluble vitamin C and fat-soluble vitamin A.

The " Fellata " colonists are better nourished than their neighbours ; they are more active and productive, and consequently able to spend more on food.

ENDEMIOLOGY.

Though no written records exist, kala-azar appears to have been fairly widespread in the Province some 50 years ago, and sheikhs and elders confirm

that whole villages were sometimes decimated and evacuated owing to its prevalence. A Government Commission under BOUSFIELD (1911) touring the area in 1909 noted the disease as increasing to a serious extent. A second Commission sent out at the end of the same year, under THOMSON (1911) saw thirty-seven cases on the Blue Nile. Cases continued to be recorded in the *Annual Report of the Director, Sudan Medical Service*, but not in great numbers, down to the present time.



ANGLO-EGYPTIAN SUDAN.

No epidemics such as those which have occurred in India and elsewhere have ever been authentically reported, but periodic increases in incidence have occasionally occurred in endemic foci of the disease. One such increase occurred during the period under review. At Dar Agil, a delapidated village of some 500 inhabitants, five cases were detected in March, 1934, followed by a further four in April. In May a complete survey of the natives was made and, by subjecting all with suspiciously enlarged spleens to splenic puncture, an additional

twelve cases were discovered. All these cases occurred in children or young adults under 20 years of age. Energetic treatment was instituted but no other preventive measure taken beyond keeping the village under careful supervision. The survey was repeated at the same time in the following year and four cases only were detected.

In other foci of infection the disease was just as prevalent in 1935 as it had been in the previous year. The disease appears to be endemic in certain villages which are recognized stopping places along the routes of communication. Most of the cases in the present series originated in some such village.

Incidence of the Disease.

(*a*) *Seasonal.*—It was impossible to deduce any definite seasonal periodicity of the disease from the vague history of the duration of symptoms given by patients on admission to hospital. Cases in the area probably mostly commenced between August and February.

(*b*) *Racial.*—The incidence of the disease in the various tribes was as follows :—

	Arab.	*Negroid.*	*Fellata.*	*Abyssinians.*
Per cent.	70	20	5	5

No particular racial predisposition can be deduced from the high Arab percentage as Arabs greatly predominate over all other races in the endemic area. The negroid incidence is low owing to their isolation and lack of communications. The Fellata possibly owe their low incidence partly to their better nutrition and partly to their unwillingness to mix with their Arab neighbours. The incidence in Abyssinian immigrants is relatively very high ; they number about 200 ragged, ill-nourished male adults, who for the most part derive a bare existence by working as water carriers, donkey boys, etc., in the larger villages. To such a degree are they attacked by the disease, that they were formerly believed to have introduced the infection from Abyssinia. Their habits, lowly mode of life and poor physique in general render them very prone to infection.

(*c*) *Occupational.*—The disease was commonly found in vagrants, cultivators, labourers, water carriers, etc.—in short, the most indigent class in all tribes. Police were sometimes infected on their tours of duty in the district, presumably by associating and living with the lowly cultivators.

(*d*) *Age.*—In the present series of cases 66 per cent. occurred in adults and 34 per cent. in children. The age grouping was as follows :

Years ..	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50
Per cent.	16	26	38	14	6



FIG. 1.—DAR AGIL. A typical kala-azar village.



FIG. 2.—KALA-AZAR HUT, showing three survivors out of a family of six children. Parents not infected.

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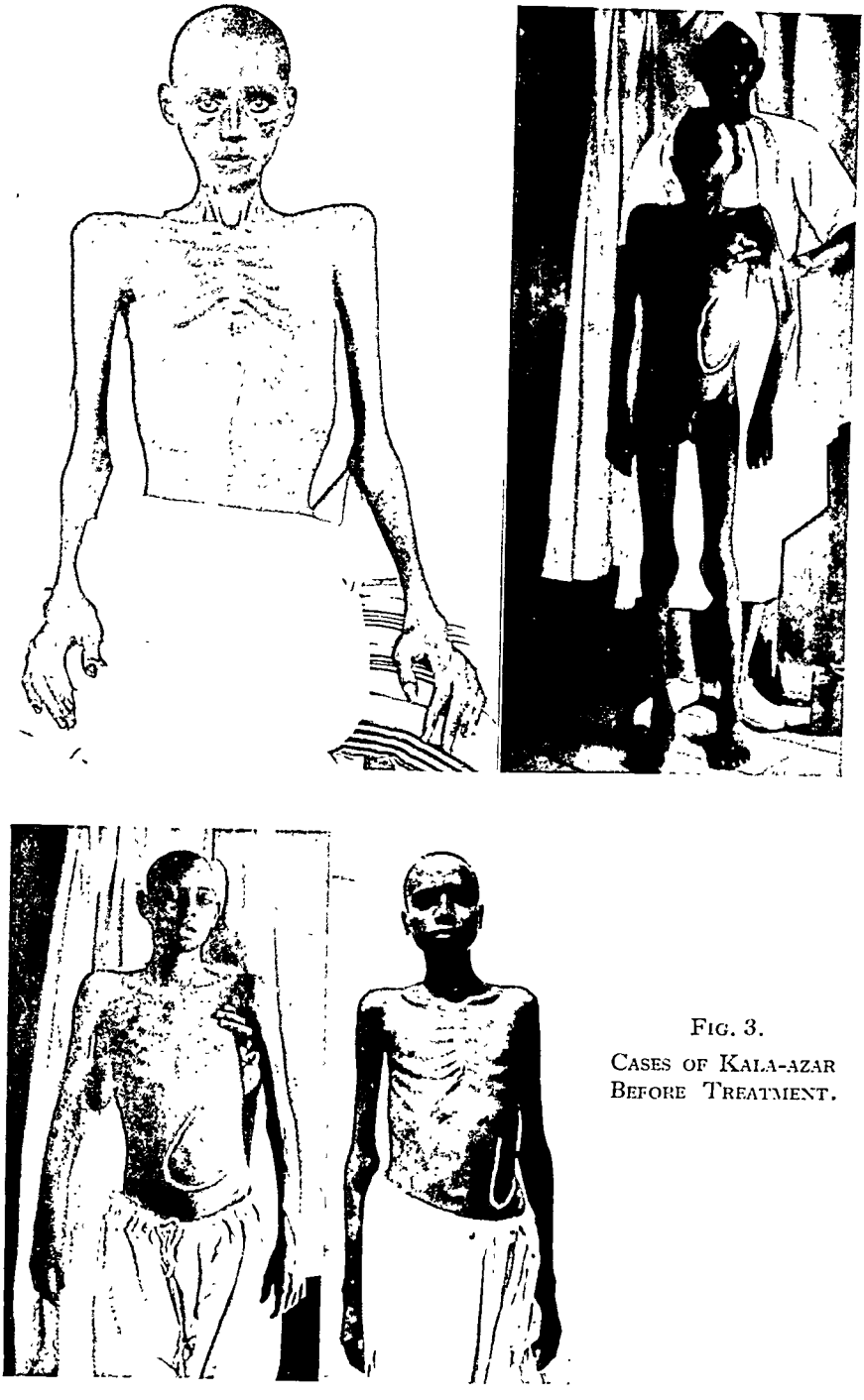


FIG. 3.
CASES OF KALA-AZAR
BEFORE TREATMENT.

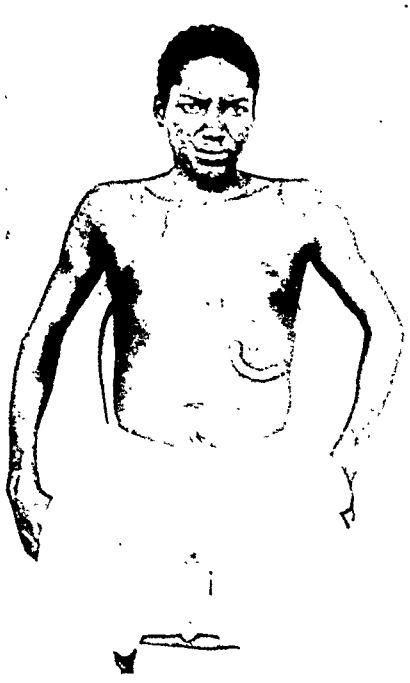






FIG. 4.
CASES OF KALA-AZAR
AFTER TREATMENT.

In children infection was commonest between the 5th and 10th year an in adults between the 20th and 25th. The youngest case treated was a boy c 18 months, the oldest a man of 47 years.

(*e*) *Sex.*—In children the disease was twice as common in boys as in girls in adults there were five men for every woman treated.

(*f*) *Familial.*—A history of pre-existing infection in the family or household was elicited in 20 per cent. of the cases treated. Considering that nearly hal of the adult males infected had no domicile at all, the actual ratio for sedentar natives was about one in three. Infection in one child was frequently followe by cases in other children in a family but rarely in adults. In several case mothers nursed sick children through the disease, but no mother in the serie became infected in this way. Two or three sick mothers had children at th breast during treatment, but in one case only could the infection possibly hav been passed on—a child of 18 months being found infected 5 months after th death of the mother from kala-azar.

The history of contact in adults was practically always contact with anothe infected adult such as husband or wife, or with someone sharing quarters. Th native habit of adults congregating in the hut of anyone seriously ill possibl accounted for the occurrence of the disease in many cases where no history o pre-existing infection could be obtained.

The period elapsing between the first and subsequent cases in a household was usually one of several months, though in a few cases it was as much as 1 o even 2 years. Amongst children, however, subsequent cases seemed to occu more quickly than in adults.

Etiology.

The diagnosis was confirmed in all cases by splenic puncture. Sometime the parasite was difficult to find and the puncture had to be repeated three o even four times. A positive result was sometimes obtained by injecting a provo cative dose of 0·2 gramme neostibosan 2 hours before puncture—a metho advocated by Napier and Gupta (1930) in blood examination.

Despite repeated examinations, parasites were found in the blood in thre cases only throughout the series. They were always scanty and phagocytose in polymorphonuclear or large mononuclear cells. Thin films only were use and no method was adopted to concentrate the cells. Marshall (1911), however engaged in similar work in the area reported the presence of scanty parasites i thirteen out of fifteen cases examined.

Following the publication of the work of Forkner and Zia (1934) on thi subject routine nasal smear examinations were carried out. Parasites were found in nine cases only out of 120 patients examined.

The parasite demonstrated by these methods was morphologically the sam as that described in India and elsewhere.

Pathology.

Postmortem examination was rarely allowed, but when possible showed, in addition to the appearances usually reported, a fairly consistent acute inflammatory condition of the upper part of the ilium often associated with a transverse ulceration of the descending colon. Parasites were never obtained from scrapings of these bowel lesions.

Skin scrapings were examined in several cases but were consistently negative.

The parasite was never demonstrated in the urine or stool of any case in the series.

Concomitant Infections.

These were present in nearly half the cases in the series.

Malaria was the commonest, and was present in 30 per cent. of cases—malignant tertian in 20 per cent. and benign tertian in 10 per cent. The superimposed malarial infection apparently frequently precipitated acute exacerbations which brought cases under medical attention. Contrary to Napier, Krishnan and Lal (1933) in the Indian type, there is apparently no antagonism between malaria and the type of kala-azar seen in this area.

Intestinal parasites were found in 11 per cent. of cases, 10 per cent. harbouring *Taenia saginata* and 1 per cent. *Ancylostoma duodenale*. The presence of these parasites undoubtedly reduced personal resistance and rendered patients more susceptible to the ravages of the major infection.

Schistosomiasis, a disease endemic in the northern part of the area, was found in 30 per cent. of children and 1 per cent. of adult cases.

Dysentery, both amoebic and bacillary, syphilis and tuberculosis were other infections occasionally encountered in routine examination of cases.

Symptomatology.

The acuteness or chronicity of onset of the disease was difficult to estimate owing to the uncertainty of individual histories. Ten per cent. of the cases gave a history of acute onset of 2 weeks or less, but on blood examination two-thirds of these showed a malarial infection which may have precipitated the acute manifestations. Twenty per cent. of the cases gave a history of 1 month's duration and a further 20 per cent. of 2 months. The numbers gradually decreased up to 6 months—few cases only giving a longer history. Two cases only claimed to have suffered from the disease for 1 year and one case for 2 years. A sudden acute onset in an uncomplicated case was extremely rare. The disease as seen in this series of cases was almost invariably of a chronic nature characterized by periodic or terminal acute exacerbations which were invariably of a febrile nature, associated with splenic enlargement, epistaxis, emaciation and anaemia, passing at a later stage to exhaustion and serious toxic manifestations.

The detailed clinical character of the commoner symptoms was as follows :

Fever.—This occurred usually in the afternoon ; the temperature rising to 103° to 104° F. and subsiding in a few hours with profuse perspiration. Though occasionally associated with headache, vomiting or even rigors, the patient did not look acutely ill during these paroxysms in the early stages of the disease, and preserved a clean tongue and good appetite. The fever was usually remittent, though occasionally intermittent or continuing for a few days on end. Four-hourly charts showed the usual double rise in nearly 80 per cent. of the cases. Exhibition of quinine before diagnosis frequently caused great variations in the temperature chart.

Splenic and Hepatic Enlargement.—The spleen was enlarged in all cases, varying in size from being just palpable to a large tumour extending into the right iliac fossa. Increase in size was not necessarily progressive nor was it intimately related to the duration of the disease. One case was admitted as a surgical emergency because the spleen had suddenly enlarged down to the level of the umbilicus and was associated with acute pain and abdominal rigidity. With two days of conservative treatment the tumour shrank to two fingers breadth below the costal margin, pain and rigidity disappeared, and puncture established the diagnosis. This was the only case of acute splenitis in the series.

Two cases complaining of only 4 days' illness had spleens of four fingers' breadth below the costal margin. As a general rule, however, cases with histories of several months had spleens down to the level of the umbilicus.

Hepatic enlargement was not such a constant or prominent feature ; it was completely absent in 11 per cent. of the series. It occurred in the more chronic type of case extending usually one to two fingers' breadths below the costal margin. In two cases only it reached the level of the umbilicus.

Emaciation and Weakness.—From the histories obtained, it appeared that these symptoms occurred very early in the Sudan type of the disease.

Haemorrhages.—Periodic attacks of epistaxis occurred in 50 per cent. of the series, some of the cases originating after treatment had been commenced. Bleeding from the gums was occasionally present. Malaena was sometimes observed usually associated with diarrhoea.

Diarrhoea.—This was a common and troublesome symptom present in 25 per cent. of the series on admission. It was often very intractable to treatment.

Other symptoms occurring less frequently were pains in the bones and joints, cough, anorexia, amenorrhoea and slight jaundice.

The pulse was always considerably accelerated and haemic murmurs were sometimes detected.

About 20 per cent. of admissions, especially those who had been acutely ill for a few weeks, showed superadded signs of toxaemia and considerable blood destruction.

Weakness was increased to the point of extreme exhaustion, lips and tongue were coated with a brown fur from haemorrhage from the gums or gangrenous ulceration in the mouth, diarrhoea was acute with blood and mucus in the stool, oedema was marked on face and ankles, and ascites sometimes present.

It was almost exclusively in this type that the earthy grey pigmentation characteristic of kala-azar elsewhere occurred.

Haematology.

An absolute reduction in all blood elements was universally present.

Red cell counts were as follows :

	1 to 2 million.	2 to 3 million.	3 to 4 million.	4 to 5 million.
Per cent.	10	40	45	5

The commonest counts were $2\frac{1}{2}$ to $3\frac{1}{2}$ million.

The most extreme degrees of anaemia were seen in the most acutely ill cases, not necessarily of long duration. The lowest count—1,400,000— occurred in an adult male with a history of 11 days' illness only.

Leucopenia was always present to the following extent :

	2,000 to 3,000	3,000 to 4,000	4,000 to 5,000	5,000 to 6,000
Per cent.	15	20	60	5

No count lower than 2,000 occurred in the series.

Differential blood counts showed the following variations :

Polymorphonuclears.—Relative percentage averaged 40 to 50 per cent. ; counts as low as 25 per cent. occurred.

Lymphocytes.—Usual average 40 to 50 per cent. Low counts of 20 to 30 per cent. frequently occurred in serious cases.

Large mononuclears.—Almost invariably increased up to 15 to 20 per cent.

Eosinophils.—Showed if anything a reduction below the normal percentage. Eosinophilia was usually present in superadded helminthic infections but rarely exceeded 10 per cent.

DIAGNOSIS.

Diagnosis rarely presented much difficulty when the disease was being keenly sought.

Large spleens associated with pyrexia which did not yield to quinine treatment were suspected and subjected to puncture. Senior native staff only were allowed to carry out the operation which was freely done both in hospital and on trek with no more preparation than a preceding blood examination. A carefully dried 2 c.c. all-glass syringe with a 22 × $1\frac{1}{8}$ in. needle was used with the usual aseptic precautions. Though over a thousand punctures were carried out, one case only possibly owed acceleration to a fatal issue to the operation—a restless moribund male at whose postmortem 2 days later were found a collection

of deeply blood-stained ascitic fluid and a small tear ¼ in. long in the splenic capsule at the site of puncture.

Junior staff in dispensaries were assisted in diagnosis by Napier's aldehyde test—a drop of 30 per cent. formalin added to 1 c.c. of serum producing well marked opacity.

Cases presenting difficulty in diagnosis were very early or very chronic infections with little splenic enlargement and only low fever. Locally occurring conditions simulated were typhoid fever, Malta fever, chronic malaria, schistosomiasis, and tuberculosis.

Course and Complications.

The course of the disease under treatment was very variable and complications frequently occurred, chiefly cancrum oris and infections of the upper respiratory passages.

Ulceration of the mouth occurred in debilitated cases and, unless strenuously dealt with, was liable to rapid extension with a fatal termination due to septic absorption. The condition occurred in 10 per cent. of the series, originating frequently after treatment had been commenced. Ulceration was found on cheek, gums, uvula, tonsil and fauces. In children in particular necrosis frequently extended to the underlying bone; in two cases recovery followed excision of a necrosed mandible.

Extension of infection to the middle ear occurred in 5 per cent. of cases. Aural discharges were frequent and occasionally complete deafness of one or both ears followed.

A virulent pneumonia sometimes supervened and was nearly always fatal.

Dysentery, usually a Shiga bacillary infection, was a very serious complication in debilitated patients and often proved fatal.

The condition of acute agranulocytosis described by Zia and Forkner (1934) occasionally occurred and was nearly always associated with ulceration of the fauces.

Two in the series died of acute intestinal haemorrhage.

Antimony poisoning occurred in several cases; its onset was usually so insidious and the symptoms so like aggravation of those of the actual disease that it was missed in several of the early cases. It occurred in the prolonged use of all antimony preparations both trivalent and pentavalent and was more frequent in adults than in children.

Prognosis.

Cases were occasionally seen who refused all treatment; these invariably died. The hopelessness of any form of treatment was often so firmly ingrained in the native mind that parents did not think it worth while bringing children to hospital, and adult cases were concealed to allow the victim to die peacefully in

his own village. Thus, in the first year of the work, treatment had to be administered under very adverse conditions, and the death-rate was as high as 50 per cent. This has been steadily reduced till in the latter part of the series it was 25 per cent.

The reduction is probably attributable to the following causes :—

1. Propaganda and actual results causing natives to seek treatment at an earlier stage of the disease.
2. Earlier diagnosis of suspected cases.
3. The ensuring of complete cure before discharge of hospital cases.
4. Increased care in the use of antimony preparations with a healthy respect for their cumulative possibilities.

Generally speaking, the earlier treatment could be commenced, the better the prognosis. Cases detected before serious clinical manifestations appeared, as in the Dar Agil survey previously referred to, practically always recovered.

During treatment a favourable prognosis could not be given unless amelioration of the condition was accompanied by shrinkage, to some extent at least, of the splenic tumour. An early permanent diminution of the spleen especially if associated with appreciable slowing of the accelerated pulse was a very good prognostic sign.

The number of parasites seen on successive splenic smears was of practically no prognostic significance.

Patients showing evidence of considerable toxaemia were very difficult to treat and frequently showed no reaction whatsoever to antimony treatment.

On the whole, children responded to treatment more favourably than adults.

Differential blood counts carried out at regular intervals during treatment suggested in 60 per cent. of the series that the relative numbers of lymphocytes present at the different counts had a certain prognostic significance. An original low count of under 30 per cent. which did not rapidly increase under treatment, or a rapid reduction of the lymphocyte count during treatment, often presaged a fatal termination.

The average period of hospital treatment in cases of recovery was 3 months—the shortest 1 month and the longest 1 year. This prolonged hospitalization compares most unfavourably with results obtained elsewhere. Though the difficulty in curing the condition was largely due to the frequently advanced stage of the disease before treatment was commenced, it would appear that the parasite in the Sudan is often peculiarly resistant to antimony therapy or that it readily becomes " antimony-fast."

Treatment.

To avoid confusing junior native medical staff, the only specific drugs used in the series were neostibosan and antimony tartrate. As soon as diagnosis was established, a course of neostibosan, administered on alternate days, was prescribed up to a total of 4·5 grammes for adults and 3 grammes for children.

The diet was made as nourishing as possible and raw or lightly cooked liver added to it. Mouth washes were given as a routine, and concomitant infections dealt with during the course of treatment.

A complete blood analysis including total and differential counts was carried out as early as possible, and repeated fortnightly during treatment. If the total red cells fell at any time to two million, a small blood transfusion of 50 to 100 c.c. was given and repeated if necessary.

On completion of the course an interval of 1 week to 10 days was allowed to elapse during which nothing was done beyond general stimulating measures. At this time an injection of Fourneau 914 often had a very beneficial effect on the general health. This interval was allowed to ensure elimination of the drug and to see if any early recurrence was likely in cases where spleens had completely disappeared under the treatment.

At the end of this period a complete re-examination including splenic puncture if possible was carried out: 60 per cent. of the " cures " were discharged at this examination. Those still positive were then given a course of 25 grains of antimony tartrate administered in the usual manner over a period of 5 to 6 weeks and re-examined after an interval as before. A further 30 per cent. of the " cures " were discharged at this stage.

Those still resistant, though in most cases showing nothing beyond a persistently enlarged and positive spleen, were subjected to a second course of antimony tartrate increased with care up to a maximum of 30 grains. Of the remainder 7 per cent. were discharged at the examination carried out after the termination of this course.

The remaining 3 per cent. were cured with a final combined course of neostibosan and antimony tartrate given alternately at successive injections until the spleen commenced to show progressive shrinkage.

Sixty per cent. only of " cures " were discharged with unpalpable or just palpable spleens. Cases were followed up as far as possible after discharge. It was frequently noted that in the size of the spleen the steady reduction, commenced during treatment, continued, and the organ was frequently unpalpable 6 months after discharge.

SUMMARY.

An account is given of the investigation of 300 cases of kala-azar in the Fung Province of the Sudan. The area is inhabited by Arabs, negroids and Fellata whose habits and mode of life are fairly primitive. Diet, though sufficient in calorie value, is ill-balanced and probably deficient in Vitamins A and C.

The disease is truly endemic and liable to periodic increase in endemic foci One such increase involving twenty-one cases is described.

Cases probably originated mostly between August and February.

There is no particular racial predisposition; individuals in a state of malnutrition and lowered resistance generally being most prone to infection.

The disease affected mainly children and young adults, and was commonest in males.

A history of contact or familial infection was common, especially in children.

Children appeared to develop the disease more quickly than adults—the usual interval being several months after exposure to infection.

The parasite was always present in the spleen; in 1 per cent. only in the peripheral blood, and in 7·5 per cent. in nasal smears. The parasite demonstrated was morphologically the same as that described in India.

On postmortem examination inflammation of the bowel was usually present.

Concomitant infections found were malaria, intestinal helminths, schistosomiasis, dysentery, syphilis and tuberculosis.

The disease was usually a chronic infection characterized by periodic or terminal acute exacerbations. The most prevalent symptoms were fever, splenic and hepatic enlargement, emaciation, epistaxis and diarrhoea.

Blood examination showed anaemia and leucopenia with a relative increase in lymphocytes and large mononuclears. Diagnosis was always confirmed by splenic puncture.

The course of the disease varied under treatment. The chief complications were septic conditions of the buccal cavity and upper respiratory passages. Pneumonia, dysentery, agranulocytosis and acute intestinal haemorrhage also occurred. Several cases of antimony poisoning occurred, especially in adults.

Prognosis depended primarily on the stage of the disease at which treatment commenced.

Ultimate cure was always associated with splenic shrinkage in addition to disappearance of all other symptoms.

Children responded to treatment more favourably than adults. Cases peculiarly resistant to antimony therapy occurred. Routine specific treatment consisted of one course of neostibosan followed if necessary by one or two courses of antimony tartrate.

Treatment was controlled by splenic puncture.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

DERMAL LEISHMANIASIS IN A NEWLY INHABITED SECTION OF ALEPPO.

BY

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During the autumn of 1935 and the winter of 1936, an outbreak of Aleppo boil occurred among a group of refugee immigrants in a settlement newly established outside the boundaries of Aleppo City. The settlers had, for the past 10 or 12 years, been living in " camps " within the city, in shacks made of packing cases and kerosene tins; but Aleppo boil had never been prevalent among them. Two months after the first settlers had moved into their new homes, however, the disease appeared among them in an epidemic form, infecting a large majority of them. In many, the lesions were multiple, and some who had had Aleppo boil in the past had it again during this epidemic. The epidemiological and clinical features of dermal leishmaniasis noted in this outbreak seem to us of sufficient interest to warrant publication.

For the last 10 or 15 years the city of Aleppo has been expanding rapidly, and new settlements are being established to provide better accommodation for the refugee settlers. These settlements are located in the uncultivated hill country to the north of the city, where, up to the time of the epidemic, no special health problems had arisen. The grounds where the epidemic started, formerly part of an orchard called " Bustan Pasha," are situated just outside the northern limits of Aleppo City and extend over an area with a dimension of about 1 km. east to west and 800 m. south to north. Late in the summer of 1935, the trees were removed, plans were made to put up about 400 brick houses,

and building began in the north-eastern section of the plot. Fig. 1 gives the plan of the settlement; the squares represent the houses which were built up

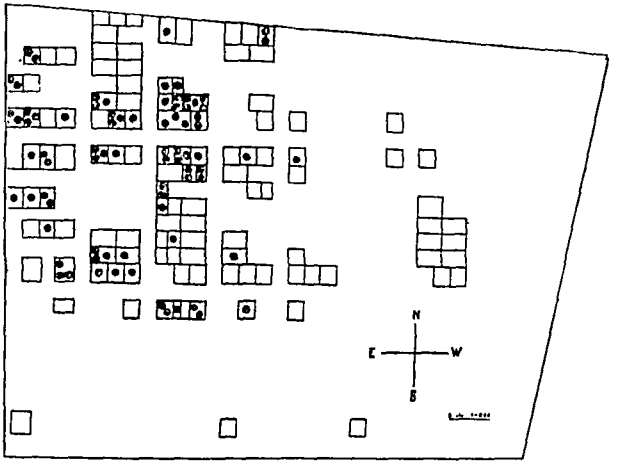


FIG. 1.—Squares denote the houses built prior to September, 1936. Black dots denote the distribution of Aleppo boil.

to September, 1936, and the dots denote the distribution of cases of Aleppo boil. Fig. 2 shows one of the newly-built brick houses. Since the building did not start until August, 1935, there was no accommodation but the bare earth

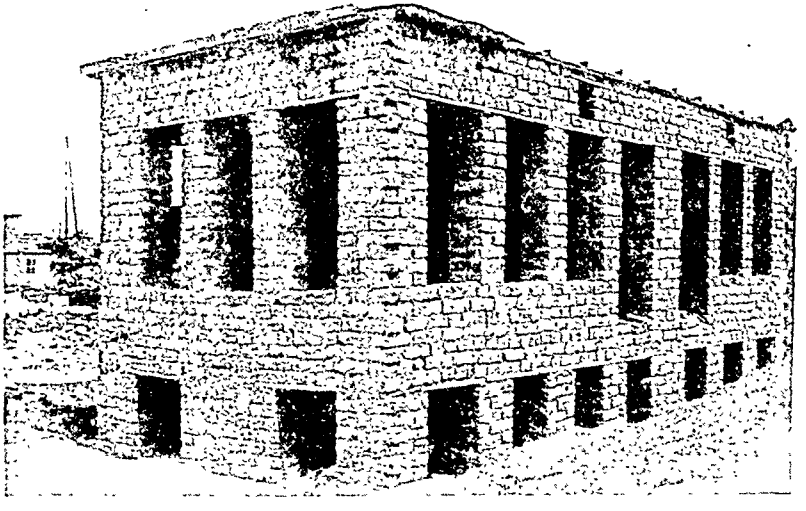


FIG. 2.—Brick houses showing construction conducive to maintenance of sandfly nuisance. Houses occupied in this state at onset of the epidemic.



FIG. 3a.



FIG. 3b.



FIG. 4.



FIG. 5.

FIG. 3a.—Eldest son before tartar emetic injections.

FIG. 3b.—Eldest son one month after the start of tartar emetic treatment.

FIG. 4.—Multiple lesions resembling dermal leishmanoids.

FIG. 5.—S.A. family (except father). The photograph is taken after a course of tartar emetic treatment, which was very efficacious in the children, but had no effect on the nodular lesions of the mother.

To face page 193.

for those who moved into the grounds earlier in the summer. The site had not, at that time, been completely cleared of vegetation ; the ground, moreover, soon became littered with rubbish and manure. Mosquitoes, fleas, flies and, especially, sandflies were able, in these conditions, to breed abundantly, affording much discomfort to those who were compelled to sleep in the open and providing a definite menace to the health of the community.

Early in October, 1935, about 2 months after the arrival of the first settlers, Aleppo boil began to appear in this new community. In order to give an idea of the mode of onset and the extent of the outbreak, we shall describe the history of the disease in the S.A. family. This is composed of five members : father, mother and three sons. The children were born in the camps, about 1 mile to the south of their present home, where the family had been living since 1922. With the exception of the father, who had had the disease in childhood and bore its scar on his upper lip, no member of the family had previously been infected. This family was one of the first to come to the locality, and began to build their house early in August, 1935. While the construction was going on, they lived in a part of the house which was dark, unplastered, and poorly ventilated, and at night suffered much discomfort from the large number of sandflies which infested the neighbourhood. In September, their eldest son, M., 9 years of age (Fig. 3*a* and 3*b*) began to show, on his face and extremities, papular lesions, which throughout the winter remained nodular. None were seen on the abdomen and back. In the spring these lesions, especially that on the nose, started to ulcerate. The family became alarmed and, in April, 1936, brought the boy to one of us (P. H.*) for examination. In the smears taken from the edges of the lesions on the nose of this case we found Leishman-Donovan bodies. On enquiry, we learned that 1 week after the appearance of the lesions in this boy, the second son, 6 years of age, had developed similar eruptions on the exposed parts of his body. When we examined the second son later in April, we found some forty papular nodules on his face, legs and forearms. Leishman-Donovan bodies were seen in smears taken from these lesions. We learned, moreover, that about the time the lesions appeared in the second son, the mother and the youngest son (3 years of age) had the same kind of multiple eruptions. The lesions of the mother and the two younger boys remained nodular, without ulceration, throughout the winter and spring. We were interested to observe that the right cheek of the youngest son was free from boils, and to learn from the mother that this boy slept always on the right side with his cheek against the pillow.

Since April, 1936, we have been making periodical visits to this settlement to obtain data concerning the extent and progress of the outbreak and to note the response of the disease to tartar emetic treatment.

*It is with deep regret that we report the death of Dr. PHILIPP HOVNANIAN in June, 1936. His son, Mr. A. HOVNANIAN, student of Medicine in the American University, Beirut, has carried on his father's part in this work.

In September, 1936, the disease had already appeared in forty-five (35 per cent.) of the 127 families who had transferred to the new locality. Detailed study of thirty-two of these families shows that, out of a total of 181 persons (ninety-seven children and eighty-four adults), seventy-eight (43 per cent.) contracted the disease. Of these seventy-eight, fifty-five (70 per cent.) were children below the age of 12. Only 30 per cent. of the adults were infected. The low percentage of adults can be explained by the fact that, out of the total number of eighty-four adults, thirty-five had already had the disease in childhood while in Turkey, and had, presumably, acquired an immunity against the disease. If the number of the adults who had had the disease is subtracted from this total number, the percentage of the incidence of the disease in the adult group is 46 per cent. It is noteworthy that, during this outbreak the disease appeared in three persons of over 60 years of age for the first time in their lives. We have also seen active lesions contracted during this outbreak in seven persons who gave a history of having had Aleppo boil in the past and who have typical scars. All of those cases who had Aleppo boil for the second time had a mild single lesion on the hand, except one who had three lesions. The lesions in all of these cases ran a shorter course than in a first infection, and in three of them healed completely within 3 months. The time which elapsed between the first and second infections in this group varied from 10 to 50 years.

As is seen in Fig. 1, the infection was most prevalent in the north-eastern section of the affected area where, in the late summer and early autumn of 1935, building first began. The detailed case histories show that the outbreak of Aleppo boil started suddenly but was of short duration, the disease being almost exclusively confined to those who had moved to their new homes in the summer and autumn of 1935. The first cases appeared in October, 1935, the majority in November and December, fewer in January and February of 1936, until, finally, only two new cases appeared after May, 1936.

It is, again, noteworthy that in some of the houses in the north-eastern section no cases of Aleppo boil were found. One particular house, although separated by a distance of only 30 m. from the main group of buildings where the disease was most prevalent, nevertheless remained free from infection. This house was inhabited by a family of six children and their parents, none of whom had previously had Aleppo boil. In spite of the fact that these people had moved to their new home as early as the summer of 1935, they lived in a well ventilated and properly plastered room on the second story where they were not disturbed by sandflies.

Clinical Observations.

Some peculiarities of this epidemic are worthy of note.

1. The lesions were multiple. The usual number of lesions was between ten and twenty, although many had from forty to eighty and some as many as

eighty-five. The disease was more widespread and severe among the children of from 4 to 10 years of age. Very few children of this age, living in the section where the disease was prevalent, escaped the infection. On the other hand, infants below 2 years of age were not afflicted so badly, probably because infants were usually protected against biting insects by a muslin cover over their faces.

2. The distribution and the appearance of the lesions, in some cases (see Fig. 4) resembled that of dermal leishmanoid. None of the cases, however, gave any history of kala-azar; and constitutional symptoms, such as chills and fever and enlargement of liver and spleen, were lacking. Blood counts in six patients who had extensive lesions showed no definite blood changes, the white corpuscles varying from 8,000 to 10,000 per c.mm. Although infantile kala-azar occurs in different parts of the Lebanon (HITTI, 1926), no cases of kala-azar in adults have been reported from Syria and the Lebanon.

3. One may assume that those who moved into these grounds about the same time and lived under similar conditions were exposed to the infection about the same time. A review of such a group of cases indicates that the lesions appeared 1 to 3 months after exposure. We have noticed that, in general, the disease was more severe and the lesions greater in number in those who seemed to have a shorter incubation period.

4. In seven cases out of a group of seventy-eight, the disease occurred for the second time. This indicates that not all persons develop a permanent immunity to oriental sore, and/or that re-infection may occur if the infective dose is massive enough! The disease, however, was very mild in those who were infected a second time, and six of these seven patients had only a single lesion.

5. The following are some observations on treatment with tartar emetic. The drug was given intravenously to thirty-five patients three times per week, the total dose varying from 12 to 30 grains according to the age of the patient. It seemed to us that, in general, the greater the number of lesions and the younger the patient, the quicker the recovery under tartar emetic. The response to treatment was very dramatic in cases with multiple ulcerative lesions. Children who had been suffering for 2 or 3 months from extensive ulcerating lesions, where the examination of the scrapings taken from the edges of the ulcers revealed Leishman-Donovan bodies in large numbers, showed marked improvement after two injections of tartar emetic, and the lesions were completely healed within 1 month. On the other hand, patients who had the nodular non-ulcerative type of lesion (see Fig. 5), and others who had only a single lesion, especially the elderly patients, did not show appreciable improvement after a course of tartar emetic.

As to the effect of tartar emetic on Aleppo boil, our observations are somewhat different from those of KHALIL (1934). Professor M. KHALIL BEY finds no Leishman-Donovan bodies in the secondarily infected ulcerating lesions and

he denies any specific action to any of the antimony preparations in different forms of dermal leishmaniasis that he has studied in Egypt.

Discussion.

In this survey we have studied an outbreak of oriental sore in a community which had been transferred to a new environment separated by a distance of about 1 mile from its previous location. The change in conditions, however, was such as to cause an immediate outbreak of the disease in the new settlement. The atmospheric conditions, the climate, the water supply, the food and mode of living remained unchanged. The state of the grounds and the type of house, on the other hand, were very different in the two localities. The kerosene tins and packing cases of which the shacks in the camps were composed may have encouraged the breeding of bed-bugs but were in no way conducive to the breeding of sandflies. The camp ground, moreover, was clean and free of any kind of vegetation. Conditions in the new settlement, however, were different. The bricks of the houses were made of straw and earth, material in which sandflies breed readily. The ground was piled with rubbish and débris and was still, in places, covered with the undergrowth of the orchard which had previously stood on that land. During the building operations of the summer and autumn of 1935, large numbers of sandflies infested the neighbourhood. As the construction progressed, however, houses were plastered and streets cleaned, and the number of sandflies became gradually less. It is significant to note that, as the improvements continued and sandflies disappeared, the outbreak of Aleppo boil subsided. In this connection it should be noted that no outbreak of this disease occurred in any of the near-by settlements, where the grounds had not been cultivated, where there was no vegetation or rubbish and where no large numbers of sandflies had appeared. It is improbable that the new settlers introduced the infection to this locality, since many of the gardeners and their families living on this ground prior to the coming in of the new settlers gave a positive history and bore the scars of the disease, which had obviously prevailed in this region in the past.

In Syria and the Lebanon dermal leishmaniasis is known as Aleppo boil. It has a patchy distribution in this country, as elsewhere. Although the disease is endemic in Aleppo, it is more prevalent during some years than others. The theory that the sandfly is responsible for the spread of oriental sore is strengthened through further evidence collected by different observers in this country who have kindly communicated their personal experiences. From Dr. A. Altounyan, who has been carrying on a wide practice in Aleppo for over 50 years, we have the information that Aleppo boil has always been more extensive and severe in those parts of the city where the hygienic conditions are poor, the streets dirty and the biting insects more abundant. Those families, moreover, residing in the cleaner quarters, who use mosquito nets or live in properly screened houses,

have either escaped the disease altogether or have had only small, insignificant lesions. Mr. J. KUENZLER, of the Swiss Mission, Beirut, who has spent more than 35 years doing hospital and missionary work in the Near East, describes similar conditions in a severe epidemic of Aleppo boil which occurred in Ourfa, Turkey, a district where the disease is endemic. During 1916-1917 there were many Kurdish and Armenian refugees in Ourfa who were brought in from the northern parts of Turkey. These people were forced to sleep in the open, having very little clothing to cover them. The epidemic was severe ; the lesions, which were scattered over the exposed parts of their bodies, were multiple, and in one patient Mr. KUENZLER was able to count as many as 260 lesions. He witnessed another epidemic where orphans, being transferred from Turkey to the Lebanon, had to spend some nights during summer in the open in districts where Aleppo button was prevalent: similar conditions were present. Mr. KUENZLER also stated that in Ourfa new cases of Aleppo boil almost always appeared during the last 3 months of the year. In the light of this observation it is suggestive that the epidemic described in this paper also began in October and reached its height during November and December. The fact that sandflies are most abundant in Aleppo during the summer and Aleppo boil only appears in autumn may be significant in determining the incubation period of the disease.

SUMMARY.

A. A sudden outbreak of dermal leishmaniasis occurred in a group of people who moved from one locality in Aleppo City to another only 1 mile to the north. These people had been living in Aleppo, in " camps," for 10 or more years. While in the camps they lived in wooden and tin shacks, had no special complaint against sandflies, and dermal leishmaniasis was not prevalent among them.

B. The new houses into which they moved were built of straw and mud bricks. The area to which they transferred was formerly a fruit orchard, the grounds of which were not completely cleared of vegetation when the new settlers came in August, 1935, and sandflies were very abundant.

C. The cases of dermal leishmaniasis were more prevalent among the people who settled in this area earlier (August to September, 1935), and very much less among those who came in later (during the winter, spring and summer of 1936) when the grounds were cleared and sandflies reduced in number.

D. This outbreak was characterized by multiplicity of lesions and the prevalence of the disease among children of from 4 to 10 years old. Seven cases were observed who suffered from dermal leishmaniasis a second time. Children and those who had multiple ulcerative lesions responded to tartar emetic. Those who had nodular or single lesions did not appreciably benefit from tartar emetic injection.

E. No such outbreak of Aleppo boil occurred in similar settlements located on small hill-tops north of Aleppo where the grounds are uncultivated and there is no vegetation, where there are no manure heaps and where no particular complaint against sandflies was made.

F. Circumstantial evidence collected in this epidemic is in agreement with the conception that the sandfly is the principal if not the only vector in transmission of Aleppo boil.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

YELLOW FEVER IMMUNE BODIES AND ANIMAL SERA.

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AND

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The simplest method of detecting the presence of immune bodies in the sera of human beings who have recovered from yellow fever consists in mixing the serum to be tested with a suspension of mouse brain infected with the neurotropic strain of yellow fever virus and injecting the mixture into the peritoneal cavities of mice which have been injected intracerebrally with a suspension of starch in saline. If the serum contains yellow fever immune bodies the mice survive, if the serum contains no immune bodies the mice die within 10 days from meningo-encephalomyelitis. This " mouse protection " test, first described by SAWYER and LLOYD (1931), has now been used in the examination of many thousands of human sera from all parts of the world and its specificity is undoubtedly very great though very occasionally there have been found human sera which neutralize the yellow fever virus when the donors of the sera cannot possibly have been suffering from yellow fever (SAWYER, BAUER and WHITMAN, 1937).

*Our thanks are due to the following for their assistance in obtaining sera : Sir JOHN MEGAW, Col. A. J. H. RUSSELL and Col. F. WARE (India) ; Dr. E. S. HORGAN, Dr. R. KIRK and Dr. S. C. J. BENNETT (Sudan) ; Mr. R. DAUBNEY (Kenya) ; Dr. W. H. KAUNTZE, Dr. H. S. DE BOER and Dr. N. J. WILLANS (Uganda) ; Dr. DAVID DUFF, Capt. J. STEWART, and Mr. G. S. CANSDALE (Gold Coast) ; Dr. T. H. DAVEY and Mr. D. H. S. DAVIS (Sierra Leone) ; Dr. F. C. MINETT (England) ; Dr. G. J. STÉFANOPOULO (France).

One of us, F.O.M., is in receipt of a full time grant from the Medical Research Council.

The mouse protection test has also been used to show the presence of immune bodies in the sera of animals which have been experimentally infected with yellow fever in the laboratory and has now been applied to the sera of animals from areas where yellow fever is known to be endemic. In endemic areas in South America (SOPER, 1936) and in Africa (FINDLAY, STÉFANOPOULO, MAHAFFY and DAVEY, 1936; FINDLAY and MACCALLUM, 1937) it has been found that approximately 20 per cent. of the sera of wild monkeys give a positive test for the presence of immune bodies to yellow fever. The sera of monkeys from Kenya where yellow fever in man is not known to occur are negative as are the sera of monkeys from India and Java. The mouse protection test was further extended to the sera of domestic and wild animals from West Africa. The sera of two sheep from the Gambia were found by FINDLAY *et al.* (1936) to give a positive protection test but the significance of this finding was uncertain as the serum from one of six English sheep also gave a " positive " test. Recently WILSON (1937) has reported that the sera of opossums from yellow fever areas in South America give positive tests.

In view of the possible rôle of animals in maintaining yellow fever infections in rural areas it appeared to be of importance to determine (1) whether the sera of wild and domestic animals, other than primates, from yellow fever areas give a positive mouse protection test and (2) the specificity of the mouse protection test when applied to such sera.

In the present communication the results of examining the sera of wild animals, other than primates, are recorded together with the findings with the sera of domestic cows and sheep.

TECHNIQUE.

The usual mouse protection test was employed, 1·5 c.c. of a 20 per cent. suspension of mouse brain infected with the neurotropic strain of yellow fever virus being mixed with 3·0 c.c. of the serum to be tested. As a routine the sera before mixing with the virus suspension were diluted 1 in 8 with saline. Six mice were used in each test and each mouse, after receiving starch intracerebrally, was injected intraperitoneally with 0·6 c.c. of the serum-virus mixture. Whenever a " positive " test was obtained, that is to say, when not more than three of the six mice died within 10 days of injection, the serum was retested and, if possible, a second sample of serum was obtained from the same animal. Sera were examined from the following wild animals :—Bush rat, *Mastomys erythroleucus* (nine); field rat, *Arvicanthis occidentalis* (one); ground pig, *Cricetomys gambianus* (two); and antelopes, various (three). These sera were obtained from Sierra Leone and the Gold Coast. The results of the tests were all negative and need not be further discussed since they agree with the negative results already obtained by FINDLAY *et al.* (1936).

Among domestic animals the sera of cows and sheep were examined.

Cow sera were examined from Sierra Leone, the Gold Coast, Uganda, the Anglo-Egyptian Sudan, Kenya, England, France and India. The results obtained with these sera by the mouse protection tests are shown in Table I: it will be seen that of 83 cow sera from Sierra Leone, Uganda and the Anglo-Egyptian Sudan 16 or 19 per cent. proved positive. These sera were all obtained from areas where human cases of yellow fever have actually occurred or in which the mouse protection test applied to human sera has given positive results. In Uganda where the percentage of human sera giving positive tests is small, the percentage of cow sera " protecting " was large, ten out of twenty-two being " positive." In Kenya, however, human cases of yellow fever have never

TABLE I.

EXAMINATION OF COW SERA FOR POWER OF NEUTRALIZING YELLOW FEVER VIRUS *in vitro*.

Country.	Number of Sera Examined.	Number of Sera " Protecting."	Number of Sera " Not Protecting."
England	119	1 (0)*	118
France	34	0	34
India	40	3 (1)*	37
Kenya	82	11	71
Sierra Leone	16	1	15
Gold Coast	15	0	15
Uganda	22	12	10
Anglo-Egyptian Sudan	30	3	27
	358	31	327

*Result of retesting a second sample of serum: in the case of the Indian bulls only two of the three positives were retested.

occurred nor has the mouse protection test applied to human sera so far given positive results: nevertheless, of eighty-two cow sera eleven were " positive." These positives curiously enough all came from the district of Lake Naivasha, which is nearer Uganda than the coastal region; no positives were obtained from the coast. The possibility that the Kenya cows had been bitten by mosquitoes or other arthropods infected with the yellow fever virus cannot be entirely excluded. In the case of the Indian cattle—Himalayan bulls—the possibility of infection with the virus of yellow fever can be entirely excluded. From two of the three Indian cattle which repeatedly gave a positive test with the first sample of serum, second specimens were obtained after an interval of

about 5 months. In the case of one bull, No. 544, the first test on the second specimen was inconclusive, three of six mice surviving, a second test negative, one of six surviving. This serum which was at first recorded as positive was therefore on further examination classed as negative. The other bull, No. 310, which had been positive with the first specimen, was also positive with the second and protected up to a dilution of one in sixty-four. Thirty-four cow sera from France were all negative. Of 119 sera from English cows 118 were negative. One serum was positive three times but a second sample obtained 5 months later was negative. This cow, " Dumpling," had lived all its life in

TABLE II.

TITRES IN WHICH COW SERA " INHIBITED " THE YELLOW FEVER VIRUS IN THE MOUSE PROTECTION TEST.*

Country.	Description of Cow.	Sex.		Dilutions of Serum.				
				1/8	1/16	1/32	1/64	1/128
England (Somerset)	" Dumpling "	F	1st sample	6	5	0	0	—
			2nd „	0	0	—	—	—
India (Himalayas)	Hill Bull 310	M	1st „	6	6	6	5	1
			2nd „	6	5	5	5	1
	„ 387	M	1st „	5	5	2	0	1
			2nd „	not available				
	„ 544	M	1st „	5	2	0	—	—
			2nd „	1	1	0	—	—
Kenya	No. 1	F		5	6	4	4	1
Anglo-Egyptian Sudan (Darfur)	M 20	F		6	6	1	0	—

*Number of mice living 10 days after intraperitoneal injection of virus and serum are recorded: six mice inoculated in each batch.

Somersetshire. Thus, of 175 cow sera from areas where yellow fever is not known to be present in man not more than thirteen sera (7 per cent.) could finally be classed as positive, and of these thirteen sera eleven came from an area in Kenya not very far removed from the Uganda border. Of seventy sheep sera from Kenya three protected. Two from Uganda were negative.

In Table II are shown the titres in which certain of the cow sera protected; the figures shown are the number of mice living after 10 days, six mice having been inoculated with each serum dilution.

The Specificity of the Mouse Protection Test for Human Sera.

The specificity of the mouse protection test for the sera of man appears to be very great. Of the many thousands of human sera that have been examined, only four have given positive tests when, as far as is known, the individuals had never had an opportunity of contracting yellow fever. These four persons with positive sera reported by Sawyer, Bauer and Whitman (1937) included one Canadian, and two adults and one child from India. The serum of the Canadian gave partial protection in three successive tests while on further examination 5 months later another specimen of serum gave two positive tests, one inconclusive and one negative. From India the serum of a 13-year-old child from Calcutta tested three times with a 10 per cent. virus emulsion gave inconclusive results twice and full protection once. A second specimen secured 8 months later, when tested against a 20 per cent. emulsion of virus, was completely negative. Two Tamil natives who had lived in the Chingleput District in Southern India all their lives also gave anomalous results. The sera of both individuals, P. B., aged 17, and N., aged 40, gave full protection on repeated tests with a 10 per cent. emulsion of virus both with undiluted serum and when diluted one in ten. P. B.'s serum tested 15 months later, against a 20 per cent. suspension of virus gave one inconclusive test and one clear-cut negative. N.'s was retested 11 months after the first testing and again a third time after an interval of 22 months. On the first occasion, in May, 1932, with a 10 per cent. virus suspension the serum protected undiluted and in dilutions of one in two and one in ten. In June, 1933, with a 20 per cent. virus suspension the serum protected undiluted and in a dilution of one in ten but gave an inconclusive result with a dilution of one in sixteen and no protection in dilutions of one in thirty-two and one in sixty-four. In April, 1935, with a 20 per cent. virus suspension, the serum protected undiluted and in a dilution of one in two, but dilutions of one in four and one in thirty-two failed to protect.

These results are not unlike those obtained with the cow sera except that the percentage of cow sera giving " positives " is considerably higher.

The Specificity of the Mouse Protection Test for Animal Sera.

The specificity of the mouse protection test for the sera of domestic animals is as yet unproven. Possible explanations for the virucidal action of cow and sheep sera on the yellow fever virus are :—

(1) Virucidal substances in varying amounts may be normally present in the sera of cows and sheep.

(2) Virucidal substances may appear under certain physiological conditions.

(3) Virucidal substances may appear as a result of bacterial or virus infections other than yellow fever.

(4) Virucidal antibodies may appear as a result of infection with the organism of yellow fever.

It is now well known that bacterial agglutinins and bactericidal substances may often be met with in normal human and animal sera, while trypanocidal substances in normal human sera have lately been demonstrated. In view of the fact that as shown by MACKAY and SCHROEDER (1936) cord suspensions of rabies and poliomyelitis viruses are completely inactivated by the addition of 40 to 50 per cent. urea solutions, it was thought that a high urea content of the serum might possibly have a virucidal effect. It was found that, as in the case of poliomyelitis and rabies virus, crystals of urea ground up with a mouse brain infected with neutropic yellow fever virus destroyed the virus, but the amount of urea which had to be added to the serum, if the virus was to be inactivated, was far greater than that present in cow sera. The urea content of " protecting " and non-protecting cow sera, very kindly estimated for us by Prof. E. C. DODDS, was in fact found to show no significant variation.

In the case of poliomyelitis, JUNGEBLUT (1935) has suggested that virucidal substances may occur under certain physiological conditions, since he has found that the sera of pregnant mares may destroy the virus of acute anterior poliomyelitis.

In the present series of cow sera " positives " were obtained from both bulls and cows, though since the English cow, " Dumpling," was " positive " shortly after a pregnancy and " negative " 5 months later there may be some connection between the physiological conditions and the presence of virucidal substances.

The fact that infection with rickettsiae produces agglutinins for various strains of *Bacillus proteus* has suggested the possibility that other diseases may possibly give rise to yellow fever virucidal substances in cow sera. In the case of human beings the production of yellow fever immune bodies by organisms other than that of yellow fever has been intensively investigated, so far with negative results: the question of cow diseases has not been so thoroughly studied. The diseases which might conceivably give rise to such a non-specific immunity in cows are contagious abortion, rinderpest, three-day sickness of cattle and Rift Valley fever. The last disease can be definitely excluded since all the sera possessing virucidal properties against yellow fever virus were tested against Rift Valley fever virus and found to have no action on this virus while cow sera protecting against Rift Valley fever virus were not virucidal to yellow fever. Many of the African cow sera were from animals which had been immunized against rinderpest so that it seems unlikely that rinderpest produces immune bodies which neutralise the yellow fever virus. The question of infection with *Brucella abortus* and with the agent, probably a virus, causing three-day sickness of cattle is receiving further study.

Finally there is the possibility that, in addition to a few cattle sera containing non-specific virucidal substances, specific immune bodies may be caused by infection with the virus of yellow fever. In order to study the reaction of the cow to the virus of yellow fever, a 2-months-old calf was injected subcutaneously

with 5·0 c.c. of serum obtained from a rhesus monkey on the first day of fever after infection with the Asibi strain of pantropic yellow fever virus. Before injection the serum of the calf was tested for its protective action against yellow fever virus by the mouse protection test and found to give a negative result. The calf showed no febrile reaction during the following 3 weeks. Blood removed 48 hours after the injection and thereafter every 2 days up to 14 days after the inoculation was injected intracerebrally in mice. Virus was present in the peripheral blood stream 4 days after injection of the virus but absent on the 6th day. A second injection of 5·0 c.c. of infected monkey serum 3 weeks after the first inoculation failed to produce any febrile reaction: the serum tested 21 days later protected in a dilution of one in sixteen. This would seem to indicate that the cow suffers a symptomless infection with yellow fever virus, although it subsequently develops immune bodies.

Discussion.

In the present state of our knowledge the results obtained from an examination of the sera of cows by the mouse protection test are difficult, if not impossible, to interpret correctly. On the one hand sera from cattle in Uganda give a high percentage of " positive " results while similar " positives " have been obtained from Sierra Leone and the Anglo-Egyptian Sudan. Cattle from these areas may conceivably have been infected by the yellow fever virus and it is possible, though improbable in view of the absence of positive human sera, that cattle from Kenya may also have been infected. The discrepancy between human and animal sera could only be explained if in East Africa the insect vectors of yellow fever were zoophilic rather than anthropophilic as in Senegal, according to Mathis (1935), *Culex fatigans* is ornithophilic rather than anthropophilic. On the other hand the cattle from India yielding positive sera could not possibly have been exposed to infection with yellow fever.

Further observations must obviously be made on the action of cattle sera on the virus of yellow fever. Whatever the results of these further observations may be they need not be taken as in any way invalidating the specificity of the mouse protection test as applied to human sera; they do, however, suggest caution in interpreting the results of the test as applied to animal sera, unless at the same time negative results are obtained with sera from the same species living in areas where yellow fever is not endemic.

Conclusions.

1. Sera from the bush rat, *Mastomys erythroleucus*, the field rat, *Arvicanthis occidentalis*, the ground pig, *Cricetomys gambianus* and various antelopes from the Gold Coast and Sierra Leone failed to show the presence of yellow fever immune bodies.

2. Of eighty-three cow sera from Sierra Leone, the Gold Coast, Uganda, and the Anglo-Agyptian Sudan, areas where yellow fever immune bodies are found in human sera, 16 or 19 per cent. neutralized the yellow fever virus in the mouse protection test.

3. Of eighty-two cow sera from Kenya where yellow fever in man is unknown, eleven were " positive " by the mouse protection test.

4. Of 153 cow sera from England and France one was " positive " but a second sample from the same cow retested 5 months later was negative.

5. Of forty cow sera from the Himalayas in India three were positive : on retesting second samples from two of these animals one serum was positive in a dilution of one in sixty-four, the other was negative.

6. Of seventy sheep sera from Kenya three protected.

7. Possible explanations of these results are discussed.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

ONYALAI: A REVIEW.

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NOMENCLATURE.

To the natives of West Central Africa the name onyalai signifies a dangerous haemorrhagic disease characterized by the formation of blood-filled bullae in the mucous membrane of the mouth and elsewhere, from which free and often fatal haemorrhage occurs. This disease, however, is known to exist beyond the confines of West Central Africa, with the result that the nomenclature of the disease is determined largely by its tribal incidence. The significance of these native names can be assessed most satisfactorily by local investigation and enquiry, hence it is not proposed to make a detailed etymological study of the nomenclature, particularly as, in a number of cases, either the clinical descriptions associated with a given native name are incomplete or else it is abundantly clear that the term employed has generic significance only, in the sense that it includes a variety of haemorrhagic diseases.

How the term onyalai is derived, or what it means, it is not possible to say; but as will be seen later it is first used by WELLMAN (1904) in his conspectus of the tropical diseases of the Highlands of West Central Africa. The name onyalai appears to be unknown to the indigenous natives of both Northern and Southern Rhodesia although they are for the most part well aware of the disease. In Northern Rhodesia GILKES (1934) states that the condition is termed *chilopa* or *akembe*. In Southern Rhodesia the term *mhuka* is used by the Mashona and *manunga* by the Matebele. It is clear, however, that whilst these terms refer to a specific haemorrhagic disease they are also employed by the native of the present day to describe practically any disease in which bleeding is a salient feature.

*My thanks are due to Dr. H. H. SCOTT, of the Bureau of Hygiene and Tropical Diseases for his generous assistance with the bibliography, to Dr. JAMES LEGGATE, of the Southern Rhodesian Medical Service, for kindly supplying me with clinical material, and to Mr. N. H. MYERS for his enthusiastic assistance with the haematological investigations.

Two other native names thought to be synonymous with onyalai are *edjuo* or *edyuo* (FELDMANN, 1905) and *kafindo* (MENSE, 1906). The name *edjuo* is first used by FELDMANN (1905) in a short article on trypanosomiasis and parotid swellings met with amongst the natives of what was then known as German East Africa. FELDMANN noted that the banana-eating natives of this area were prone to develop a parotid swelling accompanied by strabismus and mental deterioration. In some cases the syndrome was complicated by a stomatitis to which the name *edjuo* was applied and MENSE (1906) was inclined to regard *edjuo* as synonymous with onyalai. It is possible that MENSE had access to more detailed information than is contained in FELDMANN'S article since it is clear that the clinical descriptions contained in this article do not, in the writer's opinion, warrant the conclusion that *edjuo* and onyalai are one and the same disease.

MENSE (1906) mentions a condition met with in the Congo and referred to by the Unyamwezi people by the name *kafindo*. The clinical features of this disease include headache, mental depression, swollen tongue, dyspnoea, cardiac disturbances and sometimes blood in the stools. As will be seen later *kafindo* has many clinical features in common with onyalai but in the description given by MENSE there is no reference to the haemorrhagic bullae so characteristic of onyalai nor does he appear to have encountered a fatal case. MENSE himself suggests that *kafindo* is probably a manifestation of *Euphorbiaceae* poisoning. At any rate it would appear unwise to regard *kafindo* and onyalai as synonymous until more information is available concerning the former disease.

The study of the nomenclature of onyalai would appear to be still further complicated by the fact that traditional knowledge of the disease extends beyond its geographical distribution. This aspect of the problem may be illustrated by making reference to a recent paper (MATTLET, 1935). Whilst working in the Ruandi-Urundi region of the Congo, MATTLET was able to investigate a grave disease regarded by the White Fathers as well as by the local natives as *kafindo* (sometimes called *kafindo-findo* or *kapfura*). It is evident, however, from MATTLET'S studies that the natives of this area although aware of the existence of some such dread disease were poorly versed in its clinical features since MATTLET is satisfied that the cases shown him as typical examples of *kafindo-findo* were cases of cerebral infection with *Plasmodium falciparum*. The surprising failure on the part of a native population to recognize malarial infection is explained on the grounds that in this particular area of high plateaux malaria has only recently been introduced. If MATTLET'S explanation be accepted this example serves to show that considerable care and discretion are necessary in the interpretation of the natives' names of obscure diseases.

HISTORICAL.

The first reference to onyalai in English literature appears in a paper by WELLMAN (1904). In the course of a series of notes on the various diseases met

with in the Benguela district, Angola, since 1896 WELLMAN refers briefly to " a very treacherous disease " characterized by the presence of haemorrhagic bullae in the mouth and elsewhere and designated onyalai by the natives of West Central Africa. Although no statistics are given in this paper the disease was apparently of common occurrence in the Benguela district and WELLMAN appears to have been aware of its dangerous and fatal aspects. [This paper was reproduced in America in 1905 (WELLMAN, 1905.)]

A few months later in the same year MASSEY (1904) described a short series of cases of onyalai from amongst the natives of Portuguese West Africa. MASSEY also draws attention to the fact that the natives regarded the disease with fear and dread and illustrates the rapidly fatal character of the condition by referring to a robust native girl of 17 years who died the day following the appearance of the blood-filled vesicles inside the mouth.

In part justification of the belief held by the natives of Portuguese West Africa that onyalai occurred in the " far interior of Africa " MASSEY (1906 and 1907) reports a case of the disease in a native of the Baluba tribe on the Lualaba River about 10° south latitude, his earlier cases having been encountered about 13° south latitude.

WELLMAN (1907 and 1908), after describing a fatal case of onyalai, goes on to discuss in some detail the history, nature, symptoms, prognosis and treatment of the disease based on his experiences in Portuguese West Africa. WELLMAN concludes his discussion by saying that he is inclined to regard onyalai as a specific entity—a form of acute infectious disease, the cause of which has not yet been ascertained.

BALFOUR and ARCHIBALD (1908), although unable to find any records of the condition in the Sudan, drew the attention of tropical workers to the existence of this disease and stress the need for additional information.

No further references to onyalai can be traced until WELCH (1920) described a case in a member of the Wa Meru tribe who inhabit the cool foot-hills to the north-east of Mount Kenya. So far as WELCH could ascertain the disease was unknown to the natives of Kenya and as he points out in 1934 this is the first recorded case from the East African Protectorate (WELCH, 1934).

The first mention of the occurrence of onyalai in the Rhodesias is made by SCOTT (1929) whose description of the condition is based largely on notes and observations supplied by Dr. WALLACE, of the Northern Rhodesian Medical Service. The disease is described as widespread in Northern Rhodesia where, as already stated, it is designated " chilopa " or " akembe." Thus some fifteen cases were encountered annually in the Broken Hill area. Finally, GILKES (1934) gives an interesting account of his experiences of onyalai (chilopa) in Northern Rhodesia and states that the disease occurs in the villages of the Luangwa and Zambesi valleys. He is the first writer to give a systematic account of the postmortem findings but was obviously handicapped in his laboratory studies by the lack of adequate facilities. In the matter of aetiology he is inclined to

regard the disease as a non-infectious condition attributable to vitamin deficiency. The first reference to onyalai in Southern Rhodesia appears in the Report on the Public Health for the year 1924 by Dr. A. M. FLEMING, at that time Medical Director for the Colony. Commenting on the diseases peculiar to Southern Rhodesian natives, FLEMING states that one of his officers, Dr. ELLIS, reported a case of onyalai during the year, but apart from remarking on the rarity of this disease no further comment is made. In subsequent annual reports occasional references are made to the occurrence of " purpura " amongst the natives and deaths are ascribed to this cause from time to time. It is clear, however, from conversations and personal communications that medical officers in different parts of the Colony have long been aware of the existence of onyalai amongst Rhodesian natives and, as will be shown later, a most effective method of treatment was evolved by one of them.

So far as can be ascertained onyalai has not been encountered outside the African continent although a condition bearing a superficial resemblance to onyalai has been described by J. PRESTON MAXWELL (1901) from the Fokien region of South China. This condition is characterized by the formation of tough haemorrhagic bullae situated on the roof of the mouth, on the palate, uvula, inside the cheeks and in the pharynx. It is not the result of trauma but is attributed by the Chinese to the action of the web of a peculiar fly-catching spider. MAXWELL states that several of his patients assured him that they have been immediately victimised on taking condiments from a bowl from which the spider had just escaped. Apart from occasional dysphagia or dyspnoea the prognosis in this disease is stated to be good and apparently it lacks the sinister reputation that onyalai enjoys amongst the natives of Central Africa. It appears doubtful, therefore, if this peculiar disease can be regarded as synonymous with onyalai although their identity or otherwise could readily be determined by comparative haematological studies.

AETIOLOGY.

The cause of this peculiar disease is still unknown. Various suggestions have been put forward but these will be discussed later. To judge from the records of dispensaries and native hospitals males are affected more frequently than females. It is most commonly met with in early adult life, although both MASSEY (1904) and GILKES (1934) refer to cases occurring in young children. In addition most observers draw attention to the fact that the disease occurs with considerable frequency in apparently strong and robust subjects. There is a considerable amount of circumstantial evidence in support of the idea that the disease may exhibit a familial tendency, while GILKES (1934) believes that the condition may on occasion be congenital. So far as can be ascertained from a study of the literature and from personal experience cases may be seen at all seasons of the year but always sporadically. Finally, there is no evidence that the disease is either infectious or contagious.

Clinical Features.

The disease develops suddenly. The prodromal symptoms include malaise, headache, pains in the chest (Gilkes, 1934), in the muscles of the legs and in the bones (Massey, 1904). The temperature rises in most cases though not in all. In the writer's experience the maximum oral temperature encountered was 101·2° F. on the 3rd day of the illness. Gilkes (1934) points out that while the temperature seldom rises above 100° F., a temperature of 103° or 104° F. is usually of favourable prognostic significance. Fatal cases frequently show a persistently subnormal temperature. The patient soon grows listless and dull. The eyes are " heavy " and the conjunctivae suffused; the tongue is often swollen and painful and as Wellman (1908) points out the parotids may also become inflamed and tender. Anorexia is common at this early stage whilst the abdominal colic which often occurs is probably a manifestation of early bleeding into the gut. Numbness in different parts of the body is sometimes a feature.

Bleeding commonly sets in within 4 to 6 hours of these initial symptoms. In the Rhodesian cases, bleeding from the mouth and nose usually constitutes the presenting feature of the established disease, whereas in the cases described by Wellman and Massey although oral and nasal bleeding is recognized it is clear that haematuria is generally the salient clinical feature. The bleeding occurs as a sequel to the formation of haemorrhagic bullae in the skin and mucous membranes. These bullae can be studied most readily in relation to the buccal cavity where they are seen to vary in size from a few millimetres in diameter to large irregular blebs several centimetres across. The interior of the larger bullae is usually trabeculated while the outer surface shows varying degrees of umbilication. These haemorrhagic effusions consist of dark, loosely coagulated or non-coagulated blood which extend deeply into the submucous layer. The bullae so formed are found inside the cheek, on the lips, under the mucous membrane of the tongue and on both hard and soft palate. In the more serious cases bullae similar in character occur in relation to the nose, naso-pharynx, larynx, vocal cords, trachea, oesophagus, stomach, intestines and renal tract (Massey, 1904; Wellman, 1908). In addition, localized bleeding may occur into the skin and conjunctivae. It is of interest to note that the buccal lesions of onyalai although bearing a superficial resemblance to scurvy are unaccompanied by the characteristic gingival " budding " of the latter disease. Blood oozes steadily or intermittently into the mouth and nose and the breath rapidly becomes offensive.

As the disease progresses the mucous membranes grow pale and according to Welch (1920) the sclerae may become faintly tinged with yellow. The same observer states that the spleen may enlarge and extend $1\frac{1}{2}$ inches below the costal margin, but this is seldom a feature of the Rhodesian cases.

The patient grows more languid and stupid and in the severe type of case active haemorrhage occurs from the intestines and urinary tract. In some

regions, as already mentioned, bleeding from the urinary tract appears to constitute the major symptom of the disease.

Another feature of the graver type of case is the occurrence of the physical signs of bronchopneumonia probably due to the presence of small haemorrhagic areas scattered throughout the lung substance to which SCOTT (1929) has drawn attention. WELLMAN (1904) states briefly that fatal haemorrhage may occur into the brain and its membranes and again in 1908 refers to the occurrence of central nervous system involvement in three of his cases, the signs being those of acute cerebral haemorrhage with or without the characteristic oral or skin vesicles. While there is no doubt that cerebral haemorrhage may constitute the presenting symptom in onyalai it would appear unwise to attribute such haemorrhages to this disease in the combined absence of mouth lesions and confirmatory haematological data.

As evidence of the occurrence of central nervous system involvement in onyalai reference may be made to a case met with in the course of the present investigation. The patient, a native of Northern Rhodesia was admitted to the Salisbury Native Hospital in a comatose state. A massive haematoma occupied the area surrounding the right eye together with the whole of the right temporal region. No clinical history could be obtained but it was believed that the native had been the victim of a murderous assault. On careful clinical examination, however, the characteristic haemorrhagic bullae of onyalai were discovered and a haematological investigation confirmed the diagnosis. The patient died within a few hours of his admission to hospital and postmortem examination revealed a massive haemorrhage into the right frontal region of the cerebrum. Although the possibility of assault was conclusively eliminated in this instance, the possible medico-legal complications which may arise in a case of this nature are apparent.

The course of the disease is very variable. Some cases show a gentle oozing of blood from the mouth and nose for a few days with or without traces of blood in the urine. Such cases recover rapidly and apart from a mild hypochromic anaemia appear little the worse for their experience. Other cases bleed freely from the mouth, nose and urinary tract where fresh crops of bullae appear from time to time and in different situations. Severe prostration and languor accompany the attack while convalescence is slow and protracted. Others again pour blood from the nose, mouth, urinary tract and bowel so that death rapidly supervenes. In the cerebral type of the disease death may occur before external bleeding of any significance has had time to occur.

TREATMENT.

It is not proposed to discuss in any detail the various native remedies employed in the treatment of onyalai, although this aspect of the subject is not without interest. Suffice it to say that these remedies consist largely of plant

extracts usually applied externally. A plant commonly employed by the natives of Portuguese West Africa, according to WELLMAN (1908), was a species of *Albizzia* (*A. anthelmintica* A Brogn) while the Angoni natives, according to a Rhodesian authority, employ an aqueous emulsion of a bush called " mupungulila " in conjunction with the leaves of a tree called " kavunguti."

MASSEY, in his original communication of 1904, states that he gave 60 grains of sodium bicarbonate and half an ounce of unpurified cod liver oil each day internally. WELLMAN (1908) describes how he has tried quinine, various alkaline salts, oil of turpentine, acetate of lead, tannic acid, ergot and suprarenal extract but with no " perceptible results." He is inclined to think, however, that greater benefit is derived from arsenic " in full doses." WELCH (1920) treated his case with calcium chloride " in moderate doses " for 2 days followed by a tonic mixture, while GILKES (1934) recommends the following measures :— (1) Transfusion of blood ; (2) Fowler's solution ; (3) Injections of adrenalin, and (4) Calcium lactate. In addition a diet rich in vitamins is advocated.

In the writer's experience, however, the most effective treatment of onyalai is that introduced by Dr. R. M. MORRIS, of the Southern Rhodesia Medical Service, in 1926.

The treatment consists in the intramuscular injection of 18 c.c. of donor's blood in 2 c.c. of 10 per cent. sodium citrate. The injection is given into the buttocks or outer aspect of the thigh. The total number of whole-blood injections necessary varies with the severity of the case, but most cases recover after two injections while the more severe ones require as many as five or six injections.

The relapse rate associated with this line of therapy cannot be stated owing to difficulties in maintaining a satisfactory follow-up system, but so far no " return cases " have been encountered.

The foregoing treatment is sometimes supplemented by means of intravenous injections of a calcium salt but there appears to be no real necessity for this addition so far as the control of haemorrhage is concerned.

Autohaemotherapy has been employed by some workers but in the writer's experience this method of treatment has not proved satisfactory and has been discarded in favour of donors' blood intramuscularly. Dr. A. P. MARTIN, however, now Medical Director of Southern Rhodesia, states that in 1930 he successfully employed autohaemotherapy in two severe cases of onyalai. The general experience, however, is that autohaemotherapy is of little value in the routine treatment of the disease.

The value of the treatment of onyalai by means of donor's blood can be more fully assessed after it has been tried out in different regions of Africa, but there can be little doubt that it has very materially reduced the mortality rate from this disease amongst the natives of Southern Rhodesia. Some of these cases, it may be noted, came from Northern Rhodesia.

While blood transfusion is also a suitable line of treatment it seems unnecessary to resort to this more elaborate method except in those cases which

show a severe grade of anaemia together with a reticulocyte percentage of less than 5 per cent. The subsequent treatment of a case of onyalai consists in the administration of suitable haematinics together with a diet which includes adequate amounts of meat, fresh fruit and vegetables.

Prognosis.

Amongst the native population of those regions of Africa where onyalai occurs, the condition has acquired a sinister reputation and is widely regarded as a fatal disease. It can readily be understood how this conviction arose when one considers the profound effect which the sight of sudden, relentless and prostrating haemorrhage must exert on the impressionable native mind. Wellman (1904) confesses that at first he was tempted to make light of native tradition but after seeing cases die from the disease in the space of a few hours he revised his initial opinion. Writing in 1908, he states that of fourteen cases, three died; while Massey (1904) records a fatal outcome in a robust girl of 17 years who died the day following the appearance of the oral lesions. Gilkes (1934) found thirteen deaths in a series of fifty-three cases admitted to the Broken Hill Hospital, Northern Rhodesia, between 1920 and 1933; and in his own series of seventeen cases there were eight deaths. Of the seven cases studied by the writer one died from haemorrhage into the brain substance. While it is true that the native dread of onyalai is not without justification there is evidence to show that the mortality from the disease varies significantly in different districts, and according to Wellman (1908) it varies also at different seasons of the year.

Those cases in which only slight bleeding occurs from the mouth and nose usually subside spontaneously and recover completely within a few days, but where severe bleeding occurs from the mouth, bladder, bowel and elsewhere the patient is rapidly prostrated and the subnormal temperature assumes sinister significance. On the other hand, as Gilkes (1934) points out, a vigorous febrile response is usually a favourable prognostic sign. Of even greater importance, however, in the prognosis of onyalai is the promptness and thoroughness with which the " whole-blood treatment " is instituted. Since the adoption of this particular therapeutic measure the prognosis has been so improved that a fatal outcome is generally the result of gross bleeding into some vital organ such as the brain or pancreas. Recovery when it occurs is usually complete but most observers agree that there is, in some cases, a tendency for the condition to recur, although the recurrence may not take place until months or years afterwards. The study of this aspect of the problem, however, is beset with many difficulties in view of the virtual impossibility of maintaining a " follow-up " system under existing conditions in Africa.

Postmortem Findings.

Although both Wellman and Massey encountered fatal cases of onyalai no mention of postmortem findings is to be found in their writings. Brief reference to the postmortem appearances is made by Scott (1929), but the first systematic account is that given by Gilkes (1934) whose paper should be consulted by all those interested in the disease.

The writer has twice had the opportunity of examining a fatal case of onyalai postmortem and the following general account of the autopsy findings is based mainly on the notes of these cases. The body is commonly well developed and well nourished, but the visible mucous membranes are observed to be strikingly pallid. Areas of haemorrhage are commonly present in the skin and under the conjunctivae while the characteristic haemorrhagic bullae are readily demonstrated inside the mouth. The distribution and characteristics of these bullae have already been described. On opening the body cavities haemorrhagic spots or effusions are present in relation to all the serous surfaces. The pleural cavity contains a slight excess of more or less blood-stained fluid while the lung substance may be diffusely studded with areas of haemorrhage 1·5 to 2·0 cm. in diameter (Scott, 1929). In other cases the lungs show simple hypostatic congestion.. The heart may show no significant changes apart from the subendocardial purpura or there may be recognizable haemorrhages into the myocardium.

The gastro-intestinal tract presents a striking appearance. Blood, either fluid or clotted, is readily demonstrable in the lumen of the oesophagus, stomach and intestine while haemorrhagic spots or areas are seen scattered throughout the gut from the stomach downwards.

In some cases haemorrhages take place into the pancreas, liver and spleen but even more striking is the massive bilateral effusion of blood into the peri-renal tissue or into the retroperitoneal tissue extending from the lower pole of the kidney to the pelvic brim. This peri-renal haemorrhage was, however, absent from the second case examined by the writer.

The urinary tract contains a variable quantity of blood or loose blood clot while haemorrhages can be seen in the walls of the renal pelves, ureters and urinary bladder. In one case examined the postmortem findings conformed in the main to the description already given but showed in addition a massive infiltration of the tissues of the right temporal and right orbital regions together with gross haemorrhage into the anterior region of the right cerebral hemisphere. In addition the blood had passed outwards into the subarachnoid space and inwards into the ventricular system. In this instance death appears to have occurred within a few hours of the onset of the disease. (See under Clinical Features.)

Laboratory Findings.

References to laboratory findings in onyalai are few in number and those that exist in the literature throw little light on the nature of the disease. Welch (1920) mentions the differential leucocyte count in a case reported by him from the East African Protectorate but since the total leucocyte count is not given this observation is of negligible value although it is noted that no abnormal white or red cells were found.

Gilkes (1934) makes brief reference to the appearance of "secondary anaemia," the non-reduction in the haemoglobin percentage and the presence of a leucopenia associated with an increase in small round cells. In regard to the urine he noted the presence of blood and blood casts while the stools consisted either of pure blood or showed visible melaena in association with blood-stained mucus. In cases with cough and expectoration red blood cells were demonstrated in the sputum. Apart from these observations no reference to laboratory findings has been found in the literature consulted. An attempt has therefore been made to elucidate the nature of onyalai by a laboratory study of seven cases which were admitted to the Salisbury Native Hospital during the year 1935. These cases all conformed with the accepted clinical description of the established disease and may be regarded as fairly representative of the type of onyalai met with in Southern Rhodesia. One fatal case (Case VI in the Tables) was encountered in the course of this investigation.

Haematological Data.

The haematological data recorded in the sequel were obtained before systematic treatment had been instituted and represent the state of the blood after 48 to 72 hours of bleeding.

Table I.

The Red Cells in Onyalai.

Case Number.	R.B.C.'s per c.mm.	Haemoglobin per cent. (Newcomer).	Colour Index.	Size of R.B.C.'s.	Reticulocytes per cent.
1	1,750,000	36	1·0	7·48μ	0·6
2	2,520,000	54	1·08	7·32μ	6·4
3	2,240,000	48	1·09	7·32μ	1·0
4	4,640,000	90	0·98	7·32μ	2·2
5	2,840,000	50	0·89	7·17μ	1·2
6	3,430,000	77	1·13	7·12μ	3·0
7	2,860,000	43	0·74	7·20μ	5·0

Table I shows the rapid reduction in red cells which occurs within the first 2 or 3 days of the disease. As will be shown later there is no evidence of increased intravascular haemolysis, hence the rapid drop in the number of circulating red cells may be ascribed to two factors :—(1) The steady loss of blood from the affected mucous surfaces. (2) The haemorrhagic infiltration of the tissues and organs.

In the more severe degrees of anaemia an occasional nucleated red cell may be demonstrated but the characteristic feature of the Giemsa-stained blood film from a case of onyalai is the variation in the size of the red corpuscles. This feature was not subjected to statistical analysis through the medium of a Price-Jones curve but a rough estimate of the mean red cell diameter was arrived at by means of Eve's halometer. In spite of the striking degree of anisocytosis it will be seen from Table I that the red cell diameters in onyalai fall within the limits of the mean normality range. Associated with the reduction in red cells there is a parallel fall in the haemoglobin percentage, hence the resultant anaemia in a typical case of onyalai is of the normochromic type which is commonly met with in any acute haemorrhage. In spite of the anaemic state of the blood the reticulocyte count is low in the initial stages of the disease which suggests that the erythroblastic marrow is exposed to some inhibitory influence at this time. As will be demonstrated later (see Table IV) a sharp reticulocytosis occurs during recovery from the disease.

In the normal healthy person the number of platelets in the peripheral circulation ranges between 250,000 and 500,000 per c.mm. (WHITBY and BRITTON, 1935) and there is good reason for applying the same standards to Southern Rhodesian natives in a normal state of health. In onyalai, however, a most remarkable reduction of circulating platelets occurs at the onset of the disease. Whether or not the platelet reduction precedes the development of the haemorrhagic bullae is a matter for further investigation but by the time bleeding occurs the platelets have fallen far below the accepted normality range. The actual figures obtained in the course of the present investigation are given in Table II, and the technique employed in arriving at these figures was that advocated by CRAMER and BANNERMAN (1930). In some cases the platelets were so scanty that absolute accuracy in their enumeration was a matter of some difficulty and in these cases the results have been expressed as " less than 1,000 " platelets per c.mm.

Using DUKE's method it was clearly demonstrated that this striking platelet deficiency was associated with a marked prolongation of the bleeding time. The normal bleeding time in healthy natives varies between 2 and 5 minutes but in the cases of onyalai investigated the shortest bleeding time was 14 minutes and the longest 2 hours and 40 minutes. On the other hand the coagulation time invariably fell within normal limits showing that disintegration of the existing platelets occurred in a normal fashion and that no abnormality or deficiency existed in respect of the other elements concerned in the phenomenon

TABLE II.

THE BLOOD PLATELETS AND SOME PHYSICAL PROPERTIES OF THE BLOOD AND CAPILLARIES IN ONYALAI.

Case Number.	Platelets per c.mm.	Bleeding Time in Minutes.	Coagulation Time in Minutes.	Capillary Resistance Test.
1	1,750	40	3	—
2	2,100	50	3½	+
3	20,000	14½	2½	+
4	<1,000	160	3	+
5	130,640	—	—	—
6	17,150	14	3½	+
7	<1,000	42	3½	+

+ = Capillary resistance test positive.
— = No test or estimation performed.

of coagulation. In all cases the coagulation time was determined by the method of WRIGHT and COLEBROOK (1921).

The final point of interest in Table II is the positive capillary resistance test which unfortunately was not carried out on all the cases investigated. In dark-skinned natives the haemorrhagic spots of a positive capillary resistance test are often difficult to demonstrate but if the skin of the cubital fossa and wrist is previously cleared by means of clove oil the haemorrhages can then be readily seen. A positive capillary resistance test is generally regarded as an indication of some abnormality of the capillary endothelium and it is also stated (WHITBY and BRITTON, 1935) that a positive test signifies a platelet count of less than 70,000 per c.mm. It would appear, therefore, that some abnormality of the capillary endothelium may be a contributory factor in the production of the purpuric lesions of onyalai.

TABLE III.

THE WHITE CELLS IN ONYALAI.

Case Number.	Total Cells.	Neutrophils per cent.	Lymphocytes per cent.	Monocytes per cent.	Eosinophils per cent.	Basophils per cent.
1	11,400	70	22	5	3	0
2	6,800	61	33	5	1	0
3	16,600	75	19	6	0	0
4	12,400	65	28	5	2	0
5	—	—	—	—	—	—
6	7,000	46	50	4	0	0
7	6,000	68	25	4	3	0

The impression formed by a study of the white blood cells in onyalai is that a definite leucopenia exists at the time of the onset of the bleeding. The neutrophils are reduced while the mononuclears show a relative increase. The eosinophils and basophils are not affected but no doubt the white cell picture is liable to modification in the presence of coincident parasitic infection.

Table III shows a considerable variation in the total cell counts but this variation can probably be explained in terms of the duration of the illness as it is thought that a steady rise in white cells follows hard in the wake of the initial leucopenia. This aspect of the problem, however, has not been studied in detail. So far as can be ascertained abnormal white cells are not found in the peripheral circulation.

The haematological findings discussed so far refer to the early phases of the disease but in three cases it was possible to make a limited number of observations over periods of approximately 3 weeks' duration. Since the results obtained conform to a more or less definite pattern it is proposed to select one case only in illustration of the haematological changes that occur from the onset of the disease to the stage of full recovery. These results are contained in Table IV.

TABLE IV.

HAEMATOLOGICAL CHANGES FROM ONSET TO RECOVERY IN A CASE OF ONYALAI.

Date. 1935. Dec.	R.B.C.'s per c.mm.	Haemoglobin per cent. (Newcomer).	Colour Index.	Size of R.B.C.'s.	Reticulocytes per cent.	Platelets per c.mm.
2	1,750,000	36	1·0	7·48μ	0·6	1,750
3	1,500,000	33	1·1	7·48μ	4·0	106,500
6	1,950,000	40	1·0	7·64μ	20·0	229,000
8	2,310,000	44	0·96	6·64μ	26·4	231,000
10	2,700,000	50	0·92	7·48μ	22·2	—
11	2,830,000	56	1·0	7·64μ	21·4	223,000
13	2,900,000	56	0·97	7·32μ	13·0	—
16	3,220,000	61	0·95	7·48μ	5·6	264,000
20	3,420,000	67	0·99	7·64μ	2·2	—
27	4,140,000	67	0·82	7·48μ	2·4	280,000

The first two blood counts listed in Table IV were made while active bleeding was still in progress. Although 20 c.c. of whole blood had been given before the second count was made, all the subsequent observations were made after the complete cessation of haemorrhage. It is clear from this table that a steady and rapid rise in the numbers of red cells occurs immediately bleeding stops while a study of the reticulocyte percentage suggests that although some attempt is made by the erythroblastic marrow to restore the red cells during the stage of active bleeding a maximal response does not occur until some 3 to 6 days

after the cessation of haemorrhage. As is usually the case, the rise in the haemoglobin percentage lags significantly behind the rise in the red cells. Great interest naturally attaches to the behaviour of the platelets and Table IV shows that from being present in insignificant numbers at the onset of the disease they show an abrupt increase which makes itself apparent in the brief space of 24 hours. The significance of this abrupt rise will be discussed later but it is of importance to note that the rise in platelets *precedes* the cessation of haemorrhages. It is thus possible that the fall in platelets may precede the onset of haemorrhage but this hypothesis requires confirmation. The platelets continue to rise slowly and steadily after the initial increase but probably several weeks have to elapse before the platelet count is fully restored to normal values.

TABLE V.

BIOCHEMISTRY OF THE BLOOD IN ONYALAI.

Case Number.	Plasma Phosphorus.	Serum Calcium.	Urea.	Non-protein Nitrogen.	Cholesterol.	Chloride.	Sugar.
1	2·0	9·1	77	83	—	310	—
2	2·95	8·40	25·2	—	—	—	—
3	3·0	7·75	25	29	165	320	—
4	2·44	8·50	30	34	140	300	—
5	—	8·75	35	39	168	320	—
6	2·7	10·3	25	34	85	300	200
7	2·28	8·5	22	—	—	—	80

It is not proposed to discuss the biochemical findings in any detail since the data collected on this aspect are far from complete but a table has been prepared from the results obtained as they may constitute a basis for some future study or comparison. It will be noted that in most instances the serum calcium falls a little below normal values but it is not possible to regard this as a particular feature of onyalai in view of the fact that Rhodesian natives as a group tend to show low values for their serum calcium. The high urea and non-protein nitrogen values in Case 1, Table V, is explained by the presence of an acute glomerulo-nephritis while the high sugar content of the blood in Case 6 was due to an intravenous glucose saline having been given shortly before the blood was collected.

The van den Bergh reaction was carried out in four cases only but no significant results were obtained which suggests that the severe anaemic state which develops so rapidly in onyalai is not the result of intravascular haemolysis. It is probable, however, that had the test been repeated during the recovery phase an indirect reaction would have appeared in some cases as a sequel to the disintegration of effused blood.

The Wassermann reaction was carried out on the serum of four cases with negative results. This suggests that neither syphilis nor yaws is concerned with the onset of onyalai.

The Urine.

It is noteworthy that in the Southern Rhodesian cases of onyalai haematuria is a much less prominent feature than it is in the cases described from Portuguese West Africa. Indeed, it may be said that gross haematuria in a Rhodesian case of onyalai is of grave prognostic significance. The results of the urinary examinations carried out on the present series of cases are summarized in Tables VI and VII.

Table VI.

The Urine in Onyalai (a).

Case Number.	Specific Gravity.	Reaction.	Albumin.	Sugar.	Acetone.	Urobilin.
1	1,015	Alkaline	++	—	—	—
2	1,006	Acid	—	—	—	—
3	1,018	Alkaline	++	—	—	—
4	1,010	Acid	+	—	—	—
5	1,015	Acid	+	—	—	—
6	1,012	Acid	++	—	—	—
7	1,016	Acid	++	—	—	—

Table VII.

The Urine in Onyalai (b).

Case Number.	Leucocytes.	Red Cells.	Epithelial Cells.	Casts.	Crystals.	*B. haematobium.*
1	+	++	A few	Granular	Phosphate	—
2	A few	—	A few	—	—	—
3	++	+	+	—	Phosphate	+
4	A few	++	A few	—	—	—
5	+	++	+	—	Oxalate	—
6	A few	++	+	—	—	+
7	++	+++	A few	—	—	—

The Faeces.

The faeces were systematically examined for ova and protozoa and any abnormal cytological features were noted. In one case (Case 7 in the Tables) an infestation with *Taenia saginata* was demonstrated but all the others in the series appeared to be free of parasitic infection of the intestinal tract. Melaena was readily demonstrable in every case due to the swallowing of blood from the buccal and nasal lesions but in one case only (Case 6 in the Tables) was there evidence of free bleeding from the lower gut.

Discussion.

The disease which has come to be known by the name onyalai may be said to be characterized by the sudden onset of bleeding from blood-filled bullae which form mainly in relation to mucous surfaces. The bleeding which occurs leads to severe grades of anaemia and in the absence of suitable treatment, frequently proves fatal.

Various views have been expressed in explanation of this peculiar disease of African natives. Thus Wellman (1908) sums up his discussion on the aetiology of the disease by saying that he is inclined to consider onyalai a specific entity—that is to say an acute infectious disease of unknown causation. He rules out malaria and trypanosomiasis as causal factors and although he points out that certain arrow poisons and certain forms of snake bite (especially bites by the puff-adder *Clotho arietans* (Gray)) may simulate onyalai he rightly concludes that they constitute different conditions. Finally he considers that onyalai differs from essential thrombocytopenia and from what are now known as the anaphylactoid purpuras.

Mense (1906) in his discussion on the causation of kafindo suggested that the condition might be due to poisoning by *Euphorbiaceae* and other native plants but as it is not clearly established that Mense was actually dealing with onyalai this aetiological factor need not be discussed further. There is certainly no evidence in support of *Euphorbiaceae* poisoning as the cause of onyalai amongst Rhodesian natives. Gilkes (1934) is of the opinion that the disease is the result of vitamin deficiency though as he says, " there is no proof of this either in the history of cases or from the results of treatment." A similar view is expressed by Castronuovo (1934) who concludes that the condition is probably another tropical avitaminosis although neither Gilkes nor Castronuovo develops the idea further. It is recognized of course that a form of purpura (simple symptomatic purpura) does arise in association with certain fevers, in certain toxic states, *e.g.*, snake bite, and in avitaminosis, *e.g.*, scurvy. In all such instances, however, the purpuric condition occurs with slight or no deficiency in the circulating blood platelets. The essential lesion appears to

consist in damage to the capillary endothelium either in the nature of an allergotoxic phenomenon as in snake-bite poisoning or as a manifestation of some nutritional defect of the endothelium as in scurvy. Again, in simple symptomatic purpura there is no significant prolongation of the bleeding time and the clot retraction phenomenon presents no abnormalities unless for some reason an unusually severe degree of thrombocytopenia has been induced.

The nature of onyalai, however, becomes more apparent on considering the laboratory data recorded above. In the first place it may be stated that onyalai is not constantly associated with any particular form of parasitic infection. Of the seven cases subjected to laboratory investigation two showed an infection with *B. haematobium* and one with *Taenia saginata*. Repeated blood examinations failed to reveal the presence of blood parasites in any of the cases.

It has, however, been clearly established that the active phase of the disease is associated with a profound degree of platelet deficiency in the peripheral blood, so much so that in some cases it is with difficulty that an accurate enumeration of the platelets can be carried out. In support of the occurrence of this striking thrombocytopenia is the fact that the bleeding time is prolonged far beyond the limits of normality. As already stated the shortest bleeding time observed was 14 minutes and the longest 2 hours 40 minutes—a period far in excess of the normal 2 to 3 minutes. It may be said that in every instance the effect of the atmospheric temperature and the site of the puncture on the duration of the bleeding time was carefully controlled in healthy native subjects and in all cases the controls showed a bleeding time well within the normal range. The accepted explanation of the bleeding time is that given by DUKE (1915) who states that bleeding from small vessels is arrested mainly by the formation of intravascular thrombi composed of a conglomerate of platelets hence a dearth of platelets implies delay in the formation of these thrombi and therefore a prolongation of the bleeding time. Thus in onyalai the platelet deficiency is clearly reflected in a protracted bleeding time.

Further indirect evidence of platelet deficiency is to be found in the quality of the blood clot and although no special methods were employed in the study of this phenomenon, it was repeatedly demonstrated that the blood clot formed *in vitro* from cases of onyalai was soft and friable and showed imperfect syneresis. On the other hand, as shown in Table II, the coagulation time is unaffected in this disease.

One other factor remains to be considered in relation to the lesions of onyalai—namely the abnormal state of the capillary endothelium as revealed by the capillary resistance test of Hess. In this connection it may be said that while it was possible to demonstrate a positive test in five of the seven cases investigated, the purpuric lesions obtained were small and often difficult to see. This would seem to suggest that endothelial abnormality although a contributory factor is probably of secondary importance in the production of the lesions of onyalai—the thrombocytopenia constituting the primary factor.

3. The treatment of the disease is reviewed and an account given of Morris's method of treating the condition by intramuscular injections of whole blood from a healthy donor.

4. Prognosis is discussed and is shown to depend to a great extent on prompt treatment with whole-blood injections. No definite statement can be made on the question of relapses and recurrences.

5. A general account of the postmortem findings in onyalai is given.

6. The laboratory findings are discussed, the important points being (*a*) thrombocytopenia, (*b*) prolonged bleeding time, (*c*) normal coagulation time, (*d*) a positive capillary resistance test.

7. The suggestion is made, based on laboratory and clinical findings, that onyalai is an acute form of essential thrombocytopenia due to defective maturation of the megakaryocytes of the marrow with an associated modification of the capillary endothelium as a contributory cause in the production of the haemorthagic bullae.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

TREATMENT OF MALARIA WITH IMMUNE BLOOD.

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In September, 1935, we reported in the Panhellenic Congress of Salonika the first results of the treatment of malaria by injection of immune whole blood of persons living in the same area.

An account of this work, published in Greek, was later translated into English and published in London (LORANDO and SOTERIADES, 1936).

We think we ought now to give further details of this treatment, as from the beginning many experienced workers thought that either we were going to fail completely or to have only temporary results.

WORKING AREA.

We worked among 3,953 peasants living in the district of Marathon, in the Marathon Communal Hygiene Centre, established by the Greek Ministry of Hygiene and the American Near East Foundation in October, 1934.

The village of Marathon is situated 44 km. from Athens at the northern end of a plain 11 km. long and from 3 to 6 km. wide. This is the plain on which in ancient times Tetrapolis was situated (Marathon Trikorythos, Oenoi and Provalinthos) and which, after Thermopylae, was the most famous in Greece on account of the battle fought there in 490 B.C. between the Athenians and Persians. It has been practically deserted by the inhabitants because of another enemy, stronger than the Persians, malaria, which conquered the place and destroyed the population. Ancient towns were abandoned, and the visitor of to-day going through this area will see only the famous tomb on Marathon battlefield and the ruins of some of the old places, Oenoi and Ramnous, with a scattering fringe of population of those who dare to live there.

Near the ruins of Oenoi, for many years a natural spring supplied a water-mill (now abandoned) and watered the gardens on each side of the torrent

*We beg to express our thanks to Mr. LAIRD ARCHER and Miss ALICE G. CARR, R.N., LL.D., of the Near East Foundation for their valuable help.

known by the ancients as the Ravine of Oenoi. This ravine edges the villages of Marathon and Bey and goes on to the sea. Several swamps were formed near the running stream of this torrent, especially before the construction of the great dam of Marathon, built by the Ulen Company of America in 1932, on the other side of the mountain from the sea. Behind this dam collects the water of the Haradra and the Varnava, two small rivers, which is then sent to Athens through a great tunnel.

The Marathon plain is bounded and terminated on the north and north-east by the Mount Parness range of foothills. The plain itself, a narrow coastal plain, curves about the foot of these hills as far as Cape Kynos-oura to the north-east. The whole length of the plain is circumscribed by the Pentellikos foothills which run roughly north to south.

Early Effort in Malaria Control.

The first effort to control malaria in this area was made in 1907 by Professor Savas and J. Cardamatis (1908) who introduced modern methods. They found that 80 per cent. of the inhabitants were suffering from malaria and that 100 per cent. of the school children of Marathon (6 to 12 years old) had enlarged spleens. They channelled a narrow water course in the torrent of Oenoi, oiled the stagnant water every 10 to 15 days. They gave quinine prophylactically, 1 gramme per person every Saturday and Sunday. For the total period of the malaria season (*i.e.*, for the 6 months, 1st May to 1st November) they distributed 24 kg. of quinine, all of which was for 1,544 of the total 1,680 inhabitants of Marathon, Bey, Vrana and Kato Souli ; and also used some tins of petroleum. This work was not followed up after one season. Our total during the first year in which the Marathon Centre has functioned was 6 kg. of quinine for 1,763 persons, while the oil expended was 2 tons. The last season from 1st March to 31st October, 1936, required only 3 kg. 231 grammes for 3,953 persons, the oil expended in the same period being 7 tons.

Splenic Index at Marathon.

Quinine has been introduced into Greece in large quantities in the last 30 years and the spleen index of school children in many agricultural villages located near marshy places is improving. For instance, in Marathon district in 1907 the spleen index was 100 per cent. Compare that with the following years :*

1932	..	55 per cent.,	134	persons.
1933	..	39 „	120	„
1934	..	51 „	142	„
1935	..	31 „	129	„
1936	..	17 „	110	„

*Numbers given by Dr. M. C. Balfour, International Health Division, Rockefeller Foundation.

The spleen index of our group of 550 at the general clinic, Marathon Communal Hygiene Centre, which was 42 per cent. in 1934, dropped to 11 per cent. in June, 1936, in our group of 716 persons.

Species of Anopheles.

It is interesting to note that in 1907-1908 *Anopheles superpictus* and *A. maculipennis* were considered the chief transmitters of malaria, and that from 1934-1936 we found that the only mosquito with which we had to deal was *A. elutus* which had probably existed there always.* This mosquito was definitely recognized by the researchers of the Rockefeller Foundation before us (Barber, Balfour, Rice, *et al.*), and it proved to be the important transmitter of malaria in our plain.

A. superpictus breeds near Oenoi and was found in great numbers formerly ; but actually we believe that few only of the inhabitants of Marathon living near Oenoi can be bitten by this mosquito because of the construction of the dam of Marathon which has taken practically all of the water from the Oenoi torrent.

The Malaria Problem.

We cannot deal with the many phases of the work of the Near East Foundation in which we are engaged in this region but will speak only of this interesting light on malaria which has so unexpectedly come to us while pursuing regular routine work.

We note that practically all of the population of this district, men and women, work in summer time in the fields, either in tobacco production or in vineyards. Some of these people even bring their families out near the marshy places and camp for a few weeks in the summer. This is the case in Kato Souli, a village situated on the edge of a 2,000 acre swamp. It lies not far from ancient Ramnous near the Cape of Stomion or Kynos-oura and is a settlement of a few agricultural families (100 persons) where every child suffers from malaria. Adults who have lived there for years have gradually developed immunity but all their children are weak, feeble and anaemic. The spleen index of the children of this village is 100 per cent. Anopheles mosquitoes are very abundant in this settlement, among them the chief and practically the only carrier of malaria is *A. elutus*, previously described as *A. maculipennis* by Cardamatis. In 1935 and 1936, dissections proved that the sporozoite infection varies between 6 and 10 per cent. according to the month : in May we found the minimum infection, and in August the maximum.

Subtertian malaria is the commonest type of malaria, but once in a while we see cases of benign tertian. Practically everybody suffers from mixed infections in this area.

**A. bifurcatus* and *A. algeriensis* are found in this plain. They play no rôle in the transmission of malaria in Greece. *Anopheles maculipennis* is very rare in the Marathon plain. *A. elutus* breeds in the salt water marshes of the plain.

In Kato Souli 6 km. from Marathon town, no oil or Paris green was used in 1935. *A. elutus* was very abundant over the whole area where we worked. At that time only the district around Marathon town was oiled. In 1936, from 15th April to 31st October, the Kato Souli swamp was oiled as well as the rest of the district. Marathon town was anopheles-free: mosquitoes were diminished in Kato Souli. (This work was in charge of Miss ALICE G. CARR, Director of Public Health Work for the Near East Foundation, with its main centre in the Communal Hygiene Centre in Marathon.) Even after this methodic oiling some anopheles still persisted and we constantly found a high sporozoite rate among them.

New farm labourers who came from Athens, uninfected, to work in Kato Souli became infected there and suffered typical attacks of malaria. This happened also to several soldiers who were sent there to work during the malaria season.

A DEFINITION OF HAEMOTHERAPY.

After this brief introduction let us explain what we mean by haemotherapy in malaria. It is the use of whole immune blood which is injected in doses of 10 to 20 c.c. subcutaneously three or four times at intervals of a few days.

It is believed by several experts on malaria that immunity in this disease does not exist. LAVERAN (1918) stated that a first attack does not create immunity: on the contrary, individuals who had had malaria were more susceptible to it than others. He stated, however, that negroes present a greater resistance, which, however, never reaches immunity.

Experimental work on birds by E. SERGENT and BEGUET (1914) and by the SERGENT brothers (1910) found the possibility of obtaining at least what is called the prémunition stage.

Other workers such as MOLDOVON (1912), WHITEMORE, TALLIAFERO, have proved by their experiments the possibility of the existence of an increased resistance towards malaria. (See also especially THOMSON, 1933.)

EXPERIMENTAL IMMUNITY IN MEN.

Professor MARCHOUX, when working in Dakar in 1898, injected intravenously two negroes with 10 c.c. of blood containing numerous malarial parasites. Neither developed any rise of temperature (MARCHOUX, 1926).

We must mention other workers especially YORKE and MACFIE (1924) and YORKE (1926) who proved the specific immunity towards the same species of *Plasmodium* in the same race. Similar observations were made by BOYD in America (BOYD *et al.*, 1936).

In 1917 SOTERIADES used with success the blood serum of an immune individual to treat a patient with benign tertian fever. Plasmodia in crescent

form were present in the blood of the immune individual. In 1935 LORANDO and SOTERIADES reported twenty-three cases of malaria (different types of parasites) cured by using the whole blood of immune persons, injecting it into the sufferers. We can now add twenty more cases, bringing the total up to forty-three. In the present paper cases are reported which we think valuable for the discussion of this method.

CASES.

Case 1.—A.L., aged 5 years (No. 259). Spleen Hackett 2, weight 15 kg., had quinine treatment in April, 1935, 0·3 gramme daily for 10 days. But on 9th May, the fever and the positive blood (malignant tertian) proved that the case was not cured ; 14th May, the first injection of the mother's immune blood, 4 c.c., was given subcutaneously. After this injection the girl's appearance improved, spleen not palpable. On 28th May, a second injection of mother's blood was given subcutaneously plus 0·2 gramme of quinine daily for 5 days. Since then she has been repeatedly examined and always found healthy. Spleen Hackett 0, no fever, no parasites in blood. Last weight, 15th March, 1937 : 19·5 kg.

Case 2.—(No. 901), age 3 years, suffering from malaria ; with positive blood (*vivax*) on 14th May, 1935, spleen Hackett 1 ; 4·5 c.c. of mother's blood was subcutaneously injected on that date. On 27th May, a second attack of fever with positive blood (*vivax*) occurred. A second injection of mother's blood was given. Since then the child has been very well. Weight, 18th April, 1935 : 10 kg. ; 13th March, 1937 : 15 kg.

Case 4.—Cor. H. (No. 312), aged 18 months. On 15th Feb., 1935, showed mixed infection (subtertian and benign tertian). Quinine 0·3 gramme a day was used for 1 month : cure was not obtained. On 18th June, 1935, blood was still positive. On that day 10 c.c. of mother's blood was injected subcutaneously. On 20th June, patient had another attack of fever lasting 3 hours ; we gave a second injection of 10 c.c. mother's blood. One intramuscular injection of quinine 0·25 gramme was made on 22nd June by the communal doctor without our permission. No more quinine was administered. And on 25th June, a third injection of mother's blood, 10 c.c., was given. No relapse has occurred up to 15th March, 1937.

Case 5.—Pap. C. (No. 41), aged 2 years, in November, 1934, had spleen enlarged, Hackett 4. *P. falciparum* (g + rings +++), weight 13 kg. Quinine 0·3 gramme was given daily for 1 month. On 20th June, 1935, patient showed high temperature, *falciparum* rings in the blood. First injection of 10 c.c. of mother's blood was given on that date. 25th June, 1935, a second injection of mother's blood was given. Child appears clinically cured up to 15th March, 1937.

Case 9.—C.P. (No. 39), aged 13 years. This boy was first examined on 30th October, 1934. Spleen was very much enlarged, anaemia intense, obvious cachexia, as he could scarcely walk. Blood at that time was negative for malaria. He had 1 gramme quinine daily for 1 month and quinoplasmine for 5 days. On 18th June, 1935, the blood was positive (subtertian). We gave an intravenous injection of mother's blood, 10 c.c. On 2nd July and 9th July we repeated the injection. Chill and fever with parasites in the blood followed these injections.

11th July, 1935, subcutaneous injection of 10 c.c. mother's blood plus 0·5 gramme quinine for 5 days only were administered.

14th July another subcutaneous injection of 10 c.c. mother's blood was given. After this the spleen shrank from Hackett 4 to Hackett 1·5. Since that time the child has been well. He works in the fields, usually in Kato Souli where in the year 1935 *A. elutus* was very abundant and where nobody could escape being infected. This is an instructive case as it shows that immunity in this area develops very slowly and that the children have to take courses of quinine treatment every year until they are adults.

Cases 10 *and* 11 show same characteristics as Case 9. Quinine was used for a month. Typical relapses were observed. Mother's blood injected subcutaneously has brought complete cure up to the present, 15th March, 1937.

Case 12.—(No. 457) aged 10 years, resident of Athens. She moved to Marathon in December, 1934. In May, 1935, she ran a high and continuous temperature, was semi-comatose, spleen not palpable, recurrent epistaxis, all of which symptoms gave the impression to the local doctor of typhoid fever. Widal test was negative. *P. falciparum* was present in great numbers, pulse very rapid. Quinine injections were used (Dr. CAPANIDES) from 27th June to 2nd July, as well as *mother's non-immune blood*, 20 c.c. each time. (The mother had then never had malaria but later suffered from it.) From 2nd July to 9th August, the patient took 60 tablets 0·15 gramme of quinine, 30 tablets of quinoplasmine, one subcutaneous injection of 10 c.c. mother's blood. *This treatment proved ineffective* as neither quinine nor quinoplasmine nor the non-immune blood prevented a relapse from 9th to 22nd August with continuous temperature and plasmodia in the blood. (Subtertian +, tertian +++). This child moved away and has been lost to our records.

Case 17.—(No. 1,144) a child 2½ years old with tertian parasites in the blood during an epileptoid attack of malaria; 10 c.c. of blood was injected (taken from the donor Dr. CAPANIDES, the communal doctor) on the 27th of August, 1935. This injection was repeated on the 3rd of September, 1935. No other attack occurred after this treatment.

Case 18.—(No. 1,143) E.Z., 15 years old, resident of Marathon, was examined on 27th August, 1935, and malarial parasites (*vivax*) were found in the blood. The spleen was enlarged Hackett 4, haemoglobin 70 per cent., high temperature every other day.

First injection of 10 c.c. of blood was given from the donor, the resident doctor of Marathon. On the 3rd of September we repeated the injection with the same amount of blood.

The child *never used quinine* or other anti-malarial drugs. Cure maintained. Spleen, December, 1935, Hackett 1. No relapses, and is now well, 15th March, 1937.

Case 23.—(No. 484) G.D., aged 12 years. Lives out of Marathon, town of Bey. First examined on 15th December, 1934, and found anaemic with enlarged spleen—Hackett 3, febrile attacks every 8 days. This child had an *idiosyncrasy for quinine*. One tablet (0·15 gramme) caused rash, vomiting, etc. On 5th December, 1935, the spleen reached as low as the left iliac fossa. We then injected 15 c.c. of mother's blood and again 10 c.c. on the 19th of December. After these injections the child improved very much. Appetite reappeared, she felt stronger and spleen was much diminished. Up to the present date, 15th March, 1937, the child has remained well.

(No. 277) Town of Nea Makri. Sophia B., aged 15 years, weight 39 kg. First examined, 3rd March, 1936. Spleen enlarged, Hackett 2.

Fever first appeared in August, 1935, lasted 8 days. Then after 6 weeks came attacks of chills and fever. On the 9th of March, 1936, patient had a severe attack of fever, high temperature and vomiting which obliged her to come to the clinic next day for blood injection. Her blood was positive for *vivax* (rings). Spleen enlarged, Hackett 2, liver palpable under the false ribs. 10th March, 1936, first injection of mother's blood was given subcutaneously, 18 c.c.; 11th March, 1936, attack of fever lasting several hours. This girl is a shepherdess, far away in the mountains and could not come down to the clinic without great difficulty, and not unless she was ill. So she had no blood test except the first one. A second attack occurred 13th March, 1936. A second injection of 18 c.c. of mother's blood was given subcutaneously on March 17th, 1936. *Never used quinine*, never had any other attacks. On 15th March, 1937, she was found very healthy and rosy; weight, 45·5 kg.

REMARKS.

We can divide our cases into two groups: (1) Cases treated with immune blood and quinine. (2) Cases treated with only immune whole blood.

I.—CASES TREATED WITH QUININE BEFORE HAEMOTHERAPY TREATMENT.

We selected these cases purposely because one quite frequently sees these children in agricultural areas of Greece suffering from continual attacks of malaria, at least during the hot months of the year. They take quinine for several

years and some unfortunately present abscesses and fistulas from quinine injections made by inexperienced people. This condition, of course, is not observed in every child; and with quinine, atebrin and plasmoquine when methodically used Greek doctors can cure most of the cases temporarily. But every year chills and fever return until the child reaches the age of 15 years or more. Even some adults living in the same area suffer once in a while from attacks. In some cases the local doctor is practically desperate because he finds that although he uses the same treatment no cure can be obtained.

For this reason we thought of using immune whole blood for the treatment of such cases. In many cases in the beginning of our experiments we added quinine to the blood treatment for some days, as we thought that small doses of quinine for some days might help in the establishing of immunity.

In practically all of our patients we observed that the temperature dropped rapidly after the subcutaneous injection of whole immune blood, and that the spleen diminished in volume in the next few days. We failed only in two cases, as the blood we used was not immune.

Case 19.—(No. 215) aged 14 years, was examined in November, 1934. He showed enlarged spleen, Hackett 3·5, and in spite of quinine treatment suffered from malaria. He had three injections of mother's blood. But even after these injections several months later, he had some attacks of malaria. It persisted even after quinine, plasmoquine and salvarsan treatment. Puncture of the spleen was negative for *Leishmania*. The child now looked a little better (15th March, 1937); but we believe that no cure was obtained because the mother's blood was not immune. We found afterwards that the mother suffered severe attacks of malaria, though she was born and lives in the Marathon area.

Case 12.—(No. 457). We also failed to have any result with this case, as the mother's blood was not immune. The whole family suffered from malaria. Unfortunately they moved away to another part of Greece and are lost to our records.

II.—Cases Treated by Immune Blood only.

It was quite difficult to find cases of this group as almost everybody in Greece takes quinine when suffering from any kind of fever. They do this without doctor's advice. Some rare cases had not used quinine before haemotherapy, and others had an idiosyncrasy to quinine, so we were in these cases able to study the effect clearly without the interference of quinine or atebrin. We had nine such cases, with clinical cure.

Cases Treated by Immune Blood and Exposed to Reinfection.

In order to be sure that our cases are exposed to reinfection, we ought to have cases exposed to bites of artificially infected mosquitoes. This cannot be done in our area at present as no insectorium exists as yet in Greece. We know, however, that many of our cases were exposed to naturally infected mosquitoes, as they worked and lived in Kato Souli where, as we said before, nobody can at present escape malarial infection if he stays there at night.

It is interesting to note that in May, 1935, we went to Ahmed Agha, in the island of Euboea, a village on the well-known estate of Mr. Philip Noel Baker (Member of the British House of Commons). On that day we injected two

children subcutaneously with mother's blood, and one with father's blood: all were suffering from tertian malaria. The children took no quinine. We asked Mr. BAKER to report on these cases. Below is his letter of 1st January, 1937.

"When you were here in May, 1935, I made a note of three children who were very ill with malaria and whom you injected with their parents' blood. They were: George Ts., 1½ years old; Evangelos Bal., 1½ years old; Chrysanthe Bel., 3 years old.

They were all very bad cases. I thought the last was sure to die. They are now very big strong children. I have seen them all and not one of them has had a single day of fever since you injected them. We all owe you great gratitude."

We feel that we have had sufficient experience in our haemotherapy for malaria immunity to believe that there is real worth in it, and that some day we may see it properly studied by researchers who have adequate equipment for doing so. Our work at present in the Near East Foundation is not research. We caught at the hope in this treatment when we went into a very malarious region. We wanted a healthy population so that we could pursue our regular programme of rural rehabilitation. So far the treatment has certainly not disappointed us, because for 2 years treated persons have been free from malaria. The older ones are able to do their work and take their place in the community. This haemotherapy does away with expensive, long-drawn-out medical treatment; and the patient suffers from no reaction or shock.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

ACTION OF QUININE AND ATEBRIN ON THE SPOROZOITES OF *PLASMODIUM FALCIPARUM*.

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In the League of Nations report on the therapeutics of malaria* based on experimental researches it is concluded that there is no known drug which, administered in therapeutic doses before and during the early days of the incubation period, is able to suppress the infection and bring about a true causal prophylaxis.

In 1934 Colonel JAMES supplied us with a solution of atebrin and a solution of quinine base to enable us to test the action of these substances in dilutions of 1 in 1,200 on the sporozoites of the parasite of malignant tertian malaria.

We followed the technique of JAMES and SHUTE and dissected the mosquitoes on a slide in the solution of the medicament. The sporozoites suspended in 0·5 c.c. of the solution were then injected in a vein of a patient whose mental state required malaria therapy. The subsequent course of events was closely followed and controlled by clinical observation and the examination of thick blood films on the one hand and the transfusion of blood to other mental patients requiring treatment.

We give below the results we have obtained noting at once, however, the imperfections of the method which, it seems to us, cannot bring about an equal exposure to the medicament of all the sporozoites owing to the lack of uniformity in the suspension.

**Quart. Bull. Hlth. Org. L.o.N.*, 1933.

A.—First Series of Experiments.

1. The patient, Bg. Th., was given intravenously on 2nd October, 1935, a suspension, in 0·5 c.c. of a solution of basic quinine (1 in 2,500), of sporozoites from the salivary glands of sixteen anopheles infected with our routine strain of malignant tertian malaria (T.M.78). The dissection of the glands on the slide occupied over 30 minutes. The sporozoites from the different glands were not in contact with the drug for the same time but the minimum exposure was 30 minutes. The patient contracted an infection after an incubation period of 15 days, the parasites appearing on this day and fever on the following day. Quinine was thus without any action on the sporozoites. A number of other patients were inoculated with 60 to 75 c.c. of citrated blood, collected from the patient who had received the sporozoites, 4 hours, 1 day, 2 days and 3 days after the injection. The results are shown in the Table I.

It will be noted that of twelve patients inoculated with the blood of the donor only one (M.I.) became infected. This patient had received at the same time as two other patients a transfusion of blood taken from the donor 4 hours after the injection of a suspension of sporozoites in a solution of basic quinine. One has to conclude that the sporozoites were not uniformly distributed in the blood of the donor and that some at least of the sporozoites injected intravenously had not left the general circulation after 4 hours.

The negative results given by the other eleven patients confirm our previous observations which showed that the blood was non-infective for the first 3 days of the incubation period. In this case again proof that infection had not taken place was given by the susceptibility of the patients to inoculation with the same strain of malignant tertian malaria carried out 35 days later.

2. A patient (Bob I) was given a suspension of the sporozoites from fourteen salivary glands in a solution of 1/2,500 atebrin. Infection was found to have occurred on the 15th day; from this it can be concluded that under the conditions of the experiment atebrin in a strength of 1/2,500 has no action on the sporozoites and does not prevent infection.

Table II shows that the blood of a donor, taken after the same intervals of time after the injection of sporozoites exposed to quinine as in the first table, did not produce infection. Some of these patients were proved to be susceptible to the same strain of malignant tertian malaria (T.M.78) when inoculated later with either normal sporozoites or with virulent blood.

B.—Second Series of Experiments.

In these experiments we employed a more exact technique using however, the same solutions of quinine and atebrin as in the first series. As the solutions had become invaded by moulds they were sterilized before use.

TABLE I.

First Inoculation.							Reinoculation.					
Patient.	Interval in Hours between Injection of Sporozoites and Abstraction of Blood.	Quantity in c.c. of Blood Transfused.	Infection Occurring or not.	Day of Appearance of Parasites.	Day of Onset of Fever.	Period of Observation before Reinoculation.	Infection Occurring or not after Inoculation of Sporozoites.	Day of Appearance of Parasites.	Day of Onset of Fever.	Infection Occurring or not after Inoculation of Virulent Blood.	Day of Appearance of Parasites.	Day of Onset of Fever.
M.I	4	60	+	10	12							
Sp. C.	4	70	—	—	—	72 days	+	8	10			
McA.	4	90	—	—	—	72 days				+	12	20
Ruc. I.	24	60	—	—	—	71 days				+	12	14
Ojog I.	24	60	—	—	—	71 days				+	9	9
Pan. C.	24	65	—	—	—	161 days	Not reinoculated					
Pet. Ion.	48	90	—	—	—	41 days	Reinoculated but not controlled					
Fmb. O.	48	75	—	—	—	70 days	+	9	11			
Gaudr.	48	75	—	—	—	70 days				+	8	11
Vassal	72	80	—	—	—	6 months	Reinoculated but not controlled					
Munt. A.	72	80	—	—	—	6 months	Reinoculated but not controlled					
Mos. Eug.	72	70	—	—	—	69 days				+	8	11

TABLE II.

		First Inoculation.					Reinoculation.					
Patient.	Interval in Hours between Injection of Sporozoites and Abstraction of Blood.	Quantity in c.c. of Blood Transfused.	Infection Occurring or not.	Day of Appearance of Parasites.	Day of Onset of Fever.	Period of Observation before Reinoculation.	Infection Occurring or not after Inoculation of Sporozoites.	Day of Appearance of Parasites.	Day of Onset of Fever.	Infection Occurring or not after Inoculation of Virulent Blood.	Day of Appearance of Parasites.	Day of Onset of Fever.
Mlt.	4	60	—	—	—	6 months	Not controlled					
Ghrgh.	4	60	—	—	—	72 days				+	8	9
Gr. Sun	4	70	—	—	—	72 days	+	12	16			
Ghz. D.	24	75	—	—	—	71 days				—	—	—
Col.	24	70	—	—	—	6 months	Not controlled					
Andr.	24	75	—	—	—	71 days	+	12	16			
Mtr. Sp.	48	75	—	—	—	6 months	Not controlled					
Gld. I.	48	70	—	—	—	70 days	+	12	14			
D. Tun.	48	75	—	—	—	6 months	Not controlled					
H.V.	72	60	—	—	—	69 days				+	10	10
Ep.	72	80	—	—	—	6 months	Not controlled					
Es. Gr.	72	75	—	—	—	6 months	Not controlled					

Each patient inoculated received intravenously a suspension of sporozoites from a single infected gland, the dissection being carried out as before in the solution of the drug : the contact of the sporozoites with the drug was 15 minutes. Four patients received a suspension of sporozoites in a solution of basic quinine, four a suspension in a solution of atebrin, and four as controls an equivalent suspension of sporozoites in Ringer's solution.

In Table III are given the results obtained and the proof of the susceptibility of those patients which were not infected as a result of the first injection.

TABLE III.

Patients.	Suspension of Sporozoites in Atebrin 1/2000.	Suspension of Sporozoites in Quinine 1/2500.	Suspension of Sporozoites in Ringer's solution.	Period of Observation before Reinoculation.	Result of Reinoculation.
T.D.	Parasites and fever			4 months	
Bl. I.	—			76 days	Left hospital
Br. A.	—			46 days	Parasites and fever
C. Mor.	—			46 days	Parasites and fever
Asb.		Parasites and fever		4 months	
Tr. M.		Parasites and fever		4 months	
Cat.		—		4 months	Parasites and fever
N.T.		—		4 months	Parasites and fever
B.N.			Parasites and fever in 10 days	4 months	
Sp. I.			Parasites and fever in 10 days	4 months	

It will be seen from the above table that one out of four individuals inoculated with sporozoites in atebrin solution and two out of four with sporozoites in quinine solution became infected just as did the controls which received sporozoites suspended in Ringer's solution. The negative results given by the other cases bear evidence of the varying factors which may be at work such as the non-infectivity of sporozoites from certain glands or their greater sensitiveness or the variations in individual resistance of persons inoculated with sporozoites of which the vitality has been impaired.

Conclusion.

It appears from the above experiments that malarial sporozoites under certain conditions are able to withstand the direct application of quinine and atebrin in concentration of 1 in 2,500; and that an explanation of the therapeutic efficiency of these drugs based on a direct action on the parasites is excluded.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

ON DRUG PROPHYLAXIS IN THERAPEUTIC MALARIA.

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The earlier investigations of JAMES and SHUTE, YORKE and MACFIE, SWELLENGREBEL and DE BUCK* have shown that neither quinine nor atebrin is able to prevent infection after the bites of experimentally infected mosquitoes if the quantity of drug administered is not as great as the recognized therapeutic

**Quart. Bull. Hlth. Org. L.o.N.*, 1933, 2, No. 2.

dose and if it is not continued for at least 10 days after the exposure to infection, as YORKE and MACFIE have shown in the case of quinine. Experiments carried out in the same manner with plasmoquine have shown that the efficiency of this drug as a preventive agent depends on the dose (which for practical purposes is too near the toxic limit) and on the continuance of the treatment for at least 8 days after exposure to infection.

The experiments made with atebrin (0·3 gramme a day for an adult) have demonstrated the failure of this drug to act as a preventive agent when administered for 5 to 8 days after exposure to infection. The treatment however gives a latent character to the infection which does not become evident till after a very long incubation period.

The varying results obtained by different observers seem to be due to the action of the drug on the asexually reproducing forms and not to its action on the sporozoite. Actually our own observations on the infectivity of blood during the incubation period of malignant tertian malaria resulting from the intravenous injection of sporozoites has shown that an interval of 5 days occurs before the schizogonic forms invade the blood. Thus all prophylactic treatment continued beyond the 5 days is directed against these schizogonic forms of the parasite which are well known to be susceptible to the action of schizonticides.

The term medico-curative prophylaxis given by SERGENT to this type of drug prophylaxis is quite logical.

It is probable that the development of the forms intermediate between the sporozoites inoculated and the asexual forms in the blood occurs gradually. This would explain the negative results, as regards prophylaxis, always obtained when the administration of the drug is stopped before this development is complete and the positive results of YORKE and MACFIE who gave quinine in therapeutic doses during the first 10 days of the experimental exposure to infection with benign tertian malaria.

The observations to be considered in this paper have to do with forty-five individuals infected with either our strain of *Plasmodium falciparum* (T.M.78) or our strain of *P. vivax* (T.B.H.). The infection of the patients, who on account of their mental condition were in need of malaria therapy, was brought about in most cases by the bites of experimentally infected mosquitoes. A certain number were infected by the intravenous injection of virulent blood. On the day preceding the inoculation and on the 10 following days the patients received either 0·3 gramme of atebrin or 1·0 gramme of quinine hydrochloride. Controls were left untreated. The experiments were continued over a period of more than 12 months.

The results obtained are given in the table.

It appears from these results that of twelve persons, exposed to infection with the parasite of malignant tertian malaria and submitted to prophylactic doses of atebrin over a period of 11 days, not one became infected; while of

fourteen, similarly exposed to the infection and given prophylactic quinine for the same period, one alone gave any evidence of infection and then only by showing parasites without any fever after an incubation period of 46 days. Of five controls, exposed to infection but untreated, three acquired a definite infection with both fever and parasites while two became merely carriers of the parasites.

TABLE.

Type of Parasite.	Method of Infection.	Number of Patients.	Drug Used.	Days of Treatment.	Result.		
					Parasites and Fever.	Parasites Alone.	Neither Parasites nor Fever.
M.T.	M	6	A	11			6
M.T.	B	6	A	11			6
M.T.	M	8	Q	11		1	7
M.T.	B	6	Q	11			6
M.T.	M	5	controls not treated		3	2	
B.T.	M	4	A	11			4
B.T.	M	3	Q	11	1 After incubation-period of 210 days		2
B.T.	M	7	controls not treated		3	3	1

M = mosquito bite: B = injection of virulent blood: A = atebrin 0·3 gramme: Q = quinine hydrochloride 1·0 gramme: M.T. = *P. falciparum*: B.T. = *P. vivax*.

In the case of benign tertian malaria the results with atebrin were even better than with quinine. Of four patients treated with atebrin no case of infection occurred, while of three treated with quinine one became infected. Of the seven benign tertian cases left untreated as controls three showed parasites and developed fever, three showed parasites alone, while one proved immune.

CONCLUSIONS.

Our observations confirm the results obtained by other observers who have given prophylactic treatment for at least 10 days after exposure to infection. In spite of the small number of cases in our series it seems justifiable to conclude that atebrin gives more constant protection than quinine.

The prophylactic action of the drugs on *P. falciparum* infections seems to be more constant than on *P. vivax* infections.

These results are in agreement with the known facts regarding prophylactic treatment systematically carried out in malarial districts where the two parasites are responsible for natural infections.

The prevention of infection by a prophylactic treatment of 11 days, compared with our previous results obtained by a prolonged treatment during the incubation of malignant tertian malaria and the negative results of other observers who did not go beyond an 8-day course of prophylaxis, is an indication—it seems to us —that the therapeutic action is upon the schizogonic forms of the parasite and not upon the sporozoites or any intermediate stages. We mention in support of this hypothesis our observations on the lack of infectivity of the blood during the first 5 days of the incubation period of malignant tertian malaria.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

FURTHER LIGHT ON THE "YAWS-SYPHILIS" PROBLEM.

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RECIPROCAL IMMUNITY.

The fact that yaws is a disease of hot damp rural districts whilst syphilis is usually more prevalent in urban communities, seems, in a measure, responsible for the two diseases being often considered differing manifestations of the same disease. Some investigators, however, while maintaining that the diseases are distinct, hold that they are mutually exclusive; that yaws confers an immunity to syphilis and *vice versa*. Cases *A* and *B* hereunder seem to suggest that if an immunity is conferred against syphilis by the acquisition of yaws, this immunity is not absolute; mothers who have had yaws may give birth to syphilitic children several years afterwards without themselves ever having shown signs of infection with syphilis. HANSCHELL (1928) mentions a case in which a person contracted syphilis 9 years after an attack of yaws and when the Wassermann reaction was still strongly positive. It is to be noted in Case *A* that the mother gave birth to a syphilitic child (which was preceded by an abortion) 10 years after she had yaws.

The criticism that may be applied to most cases reported in the literature of infection with syphilis subsequent to infection with yaws is that the degree of immunity possible, whether to yaws or to syphilis, had not developed in the time elapsing between infection with yaws and the contraction of syphilis. This criticism hardly seems to apply to Case *A* or to that reported by HANSCHELL.

Regarding the immunity to yaws, conferred by syphilis, TURNER (1936) states that syphilis confers an immunity to yaws which is as great as, if not greater than, that conferred by yaws itself. He previously pointed out that within the first 3 years after infection with yaws, re-inoculation with heterologous strains of yaws spirochaetes may give rise to a modified or abortive attacks of yaws, but that after a period of 10 years the majority of yaws-infected persons are refractory to re-inoculation.

In the following two cases a previous infection with yaws did not prevent two mothers from giving birth to children with signs of congenital syphilis. It is worthy of note that it was evidence of syphilis in the children which first led to the suspicion that the mothers had been infected with syphilis. These cases were seen in rural parts of Jamaica, where the incidence of syphilis is negligible.

YAWS NOT HEREDITARY.

It is generally accepted that yaws is not hereditary. On this point most writers seem to agree—ROGERS and MEGAW (1930), HERMANS (1931) who also quotes BAERMANN and HALLENBERGER (1916), STANNUS (1935) who also quotes MARTLETT (1933), MOSS and BIGELOW (1922), MAXWELL (1839). Wherever doubt has been thrown upon the non-hereditary nature of yaws, the source of information has been found to come from districts where there were cases of both yaws and syphilis, and with insufficient proof that the disease transmitted was yaws and not syphilis. On the other hand, in areas where syphilis is said to be unknown, observers in Jamaica and elsewhere seem all to agree that yaws is not hereditary. (STANNUS, 1935, quotes VIGONI and PURCELL on this point.)

Yaws, also, does not give rise to any congenital stigmata corresponding to Hutchinsonian teeth, bossing of frontal bones, iritis, etc. In a total of over 6,500 histories and examinations of yaws cases investigated, among parents and children in rural parts of Jamaica, no case met with has given any real evidence that yaws gives rise to congenital lesions or stigmata. Cases *A* and *B* are the only two instances met with that would have thrown doubt on this statement. Physical examinations and blood tests of six infants, born to mothers who received no form of treatment for yaws lesions present during the period of gestation, were quite negative. Two of the children were born within 2 years and 5 years respectively of the mothers' infection. With the other four children a very much longer time had elapsed between the dates of the original infection of the mothers and the relapsing lesions which were present shortly before the birth of the children. In Case *C* hereunder, the mother became infected with yaws in the later months of her pregnancy. The disease process remained active for the rest of the pregnancy and after the birth of the child. Nevertheless, a healthy, alert child was born with neither stigmata nor signs of yaws and with negative serological (Wassermann reaction) findings.

CASE A.

C.W., mother, 29 years of age ; born April, 1908 ; seen 7.4.37.

History.—She gave a history of yaws infection at 9 years of age—1917. The primary lesion was on the left leg. A fine scar is still visible at the site. This lesion was followed shortly by a secondary eruption. She received no specific treatment for the infection.

Pregnancies, etc.—In September, 1925, when 17 years old, she met " X " a man between 30 and 40 years of age with whom she began to consort. This man was her first lover, she stated. In October, 1925, 1 month later, she had a profuse leucorrhoeal vaginal discharge, but did not notice any abrasions or sores on the genitalia or other parts of the body. In December, 1925, 3 months later, she noticed an eruption on her thighs and legs. The eruption she described was of the nature of tiny bumps, some being quite small while others grew larger ; a few broke down into small sores. The latter lesions were some time in healing, and left fine scars which she was able to point out. She did not seek medical treatment for the lesions. They were quite gone before March, 1926 (7 months after she began to associate with " X "). Whether this eruption was due to infection with syphilis or yaws or some other skin condition, the main issue, *i.e.*, a mother giving birth to a syphilitic child 10 years after infection with yaws, remains unchanged.

She became pregnant for " X " in March, 1926, but in May, 1926, aborted. In September, 1926, she was again pregnant and gave birth on 31st May, 1927, to Hazel, a syphilitic child, whose history is stated below. There were no further abortions or miscarriages, but three other children were born on 15th June, 1928 ; 28th January, 1930, and 27th November, 1931, respectively. These last three children when seen in 1937, looked healthy and showed no stigmata of lues. No serological tests, however, were done.

In 1936, C.W. developed an ulcer the size of a two-shilling piece over the right external malleolus, for which she received six intramuscular injections of a bismuth preparation, in January and February, 1936. The ulcer did not heal until August, 1936, and that following a change in local treatment. The ulcer recurred in November, 1936. It was still present in April, 1937, but Wassermann reaction tests before and after a provocative injection of neo-arsphenamine were then negative.

When questioned as to the abortion, she admitted having had a slight fall shortly before, but asserted that more severe falls had occurred during other pregnancies without mishap.

On physical examination, C.W. had an ulcer over the right external malleolus which showed some response to a few injections of bismuth salicylate intramuscularly. There was a typical scar over the alleged site of the primary yaws lesion. Other fine scars, already referred to, were on thighs and legs. There was also a firm contracted scar over the left tendo-achilles, seemingly the result of an infected blister she had when 7 years old. There were no other abnormal findings, nor were there any in the cardiovascular and nervous systems.

" X"—Father of children mentioned above.

History : (As obtained from C.W.).—" X " arrived from a part of the island where there is no yaws and took up residence in the district where C.W. lived. Before and after his first association with C.W., he used to make frequent trips to the city of Kingston (a seaport) to sell provisions. According to his paramour (C.W.) he would not be above consorting with other women on his trips to the city. He had a former paramour and was also in close association with another woman while she was his paramour.

" X " died in February, 1931, after an illness lasting 6 months. He first complained, C.W. stated, of a pain as if being pierced by a needle in one knee, and later complained of abdominal pains. Before he died he had to be lifted on and off his bed. She was able to throw no further light on his previous history.

Hazel.—Female, 10 years of age ; born 31st May, 1927 ; weight 52 lb.

Complaint.—Child is backward at school and not thriving.

Previous History.—Conjunctivitis at birth. Had " snuffles " when 1 month old. The mother experienced much difficulty in getting her to feed at the breast. Snuffling

improved when the child was 2 years old. Had an injury to her right elbow as a baby. No previous history of yaws or of skin lesions suggestive of the disease. She had chicken-pox in March, 1937. No other illnesses of note, but has never been strong, alert or bright, her mother claims.

Physical Examination.—General condition: Undersized for age. Looks dull. Is mentally slow.

Skull bones: Slight bossing of frontal bones.

Eyes: Right corneal opacity. Is unable to see at all from this eye. Left: Pupils circular, equal, react to light and accommodation. No iritis.

Teeth: Typical Hutchinsonian teeth, upper and lower central incisors.

Nose: Bridge of nose seems rather depressed even for a negro child.

Mouth and pharynx, skin and mucous membranes, long bones, nervous system: No abnormal physical findings.

Heart: Apex within mid-clavicular line; sounds regular, pure, not accentuated.

Serology: Wassermann reaction 4–4.

Response to Treatment: After one course of bismuth salicylate and one of neo-arsphenamine the patient looked definitely brighter and more alert.

Note.—A period of 10 years had elapsed between the mother's infection with yaws (1917) and the birth of the child (1927). The chances of the mother transmitting yaws to her child after such a period must be considered remote even if the disease were hereditary. She had received no treatment during that time which could have interfered with the course of the disease or the development of immunity; but between September, 1925, and the birth of the child in 1927, it does appear that the mother was exposed to infection not only with gonorrhoea but also with syphilis. It is suggested, after weighing the available evidence, that she had yaws in 1917, gonorrhoea in 1925 and syphilis in 1925 or 1926. The short course of bismuth therapy which the mother received in 1936 and lapse of time may have contributed to, if they were not fully responsible for, the negative serological findings in 1937.

CASE B.

D.H., mother, 17 years of age; seen September and November, 1936; also March and April 1937.

History.—She gave a history of a first attack of yaws in 1934 when 15 years of age. The initial lesion occurred on the left internal malleolus. This was followed by secondary macular lesions on face and extremities; later by secondary plantar lesions. She received a few intramuscular injections for the lesions in 1934. Then, in February and March, 1935, and again at the beginning of 1936, she received further intramuscular injections for relapsing plantar lesions. The lesions recurred, however, in July, 1936. When she was seen in September, 1936, there were non-ulcerative plantar lesions of yaws on both soles and one ulcerative plantar yaws papule. For these she received no further treatment. She was visited in November, 1936, when the plantar papule was found to be healed, although the non-ulcerative plantar lesions were still present.

She admitted that sexual intercourse took place for the first time in July, 1935, when she was 16 years of age, with a young man 18 years old. Pregnancy resulted and there was a miscarriage in the fourth month of her pregnancy, but there was no history of skin rashes or genital lesions of any kind.

In February, 1936, she met U.V. (whose history is given hereunder) and had sexual intercourse with him. He was her second consort. She became pregnant for this man in March, 1936. She gave no history of a vaginal discharge or of skin rashes or evident abrasions on the genitalia following intercourse with U.V., with the exception of the plantar lesions of yaws already mentioned as recurring in July, 1936.

She gave birth to a boy on 26th December, 1937. Three months later mother and child were seen. The non-ulcerative lesions on the mother's feet were just about clear. There were no definite stigmata to be observed on the child but he was thin and puny and evidently not thriving. In addition, the Wassermann reaction of the child's blood was strongly positive.

U.V., father of child ; aged 35 years ; seen 5th April, 1937.

History (as given by himself).—He had lived from babyhood until September, 1928, in a rural district where the disease of yaws is unknown. In September, 1928, he went to Kingston, a seaport city, and remained there until August, 1930. He came to reside in his present district, where D.H. lived, in 1930. He had lived with a former paramour between June, 1931, and June, 1933, when they parted. He had one attack of gonorrhoea in 1931 and another in 1932. In November, 1935, he noticed a small penile sore which grew to the size of a shilling piece. This lesion was not completely healed, in spite of constant dressings, until March, 1936. He did not, however, place any serious import on the lesion and did not seek medical attention. He first consorted with D.H. on the 22nd of February, 1936. He stated he never had yaws or injection treatment.

Physical Examination.—U.V. is an intelligent man, well built and well nourished. A fine circular scar, about the size of a shilling piece, was just perceptible on his prepuce (antero-laterally towards the right).

All other physical findings appeared to be normal.

Discussion.

Some degree of immunity may have developed in the mothers of the two syphilitic children to account for the absence of symptoms and signs of the disease in the mothers. It is well known, however, that the birth of a syphilitic child may be the very first reason for suspecting a mother to have been previously infected with syphilis. The writer has seen a case with Hutchinsonian teeth, bossing of frontal bones and involvement of the eighth nerve, leading to deafness, in a girl child born to a woman whose only sign of having had syphilis was a positive Wassermann reaction. This woman gave no previous history of yaws and she never suspected that she had ever had syphilis.

It is likely that there may be some degree of reciprocal immunity between yaws and syphilis since both diseases produce the same types of serological reaction and consequently should stimulate similar defence mechanisms in the body. Any lesions, however, which may occur several years after infection with yaws and which can be simulated by syphilis in its protean manifestations, could only be ascribed to yaws after a meticulously careful history has been taken to exclude subsequent exposure and possible infection with syphilis and the possibility of late manifestations of hereditary syphilis.

CASE C.

B., mother, 21 years of age ; seen October and November, 1936, and in March and May, 1937.

History.—She gave no history suggestive of yaws or of syphilis prior to 1936. She became infected with yaws in an injury on the right thumb in August, 1936. The source of infection was, apparently, her little boy who had caught yaws from children in a neighbouring home and whose lesions the mother would bathe each morning.

She was then 5 months pregnant. When seen in October, 1936 (7th month of pregnancy) the mother had five typical framboesiform lesions on her face with others on the neck and upper chest (dark field positive to *Spirochaeta pertenue*). The primary lesion had healed over. There were no other lesions evident, nor were there any on the palms and soles of the feet. She received an intramuscular injection of bismuth salicylate (3 c.c. of a 10 per cent. solution in olive oil) in October but did not attend for further treatment.

She was visited in November, 1936, but given no further treatment. Most of the lesions were much improved and covered with dried crusts which were being shed. A papule situated on the upper lip, at the vestibule of the nares, did not, however, seem to be receding.

A well developed male infant was born on 15th December, 1936.

Mother and child were again visited towards the end of March, 1937. On examination the papule on the mother's lip had become a typical framboesioma 1 inch in diameter; the soles of her feet were almost covered with the typical epidermal hyperkeratosis and stripping of non-ulcerative plantar yaws; in addition there were a few fissures on the soles of her feet, a condition not infrequently found accompanying plantar lesions in yaws and which is considered infectious. The infection must, therefore, be considered to have retained its activity throughout the later months of the pregnancy and subsequent to the birth of the child. (The spread of the disease to the soles of the feet is considered to have taken place haematogenously). However, the infant looked healthy, well nourished and bright. On examination no stigmata, skin or bone lesions were found. In addition, the Wassermann reaction of his blood was negative. When it was mentioned to the mother that it was a wonder she had not already infected her infant with yaws from the lesion on her lip, she replied that she was careful and never kissed the baby.

The husband, who was also seen in November, 1936, was found to be infected with yaws. His primary lesion occurred in an injury on the extensor aspect of his left elbow which he received in September, 1936. He gave no history suggestive of an infection with yaws or syphilis before September, 1936.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

THE VIRULENCE OF *TRYPANOSOMA RHODESIENSE* IN RELATION TO CYCLICAL PASSAGE THROUGH *GLOSSINA MORSITANS*.

BY

J. F. CORSON, M.D.

There has been much discussion about whether cyclical passage through tsetse flies has any influence on the characters of polymorphic trypanosomes and a few experiments have been made with results that have varied with different workers and in different experiments by the same worker. Reference is made here only to changes in the virulence of trypanosomes. SCHILLING and SCHRECK (1930) found in 1914 that an old laboratory strain of *Trypanosoma brucei* showed a great loss of virulence after a single passage through *Glossina morsitans*. LESTER (1932) found that a single passage of a serum-fast strain of *T. brucei* from a guineapig through *G. tachinoides* resulted in a great loss of virulence for guinea-pigs and mice. In a later paper (LESTER, 1933) he referred to work by TAYLOR and MACKENZIE who found that an old London strain of *T. rhodesiense* became greatly reduced in virulence after a single passage through *G. tachinoides*, while with a human strain, Ayu 5, there was no well-marked change after cyclical passage. LESTER also found that another strain of *T. brucei* showed only a slight loss of virulence after a single passage through *G. tachinoides*.

In experimental work with a strain of *T. rhodesiense* and a strain of *T. brucei* I did not find that the virulence was less after cyclical passage through *G. morsitans*, but it was only in the case of the *T. brucei* strain that the virulence had previously become greater during maintenance by direct inoculation. The strain of *T. rhodesiense* (CORSON, 1932) was inoculated from a man into sheep and goats in March, 1930, and was maintained in them by inoculation for about 19 months. In 1930 and 1931 rats which were inoculated from the sheep and goats lived for from 20 to 50 days. In December, 1931, *G. morsitans* were infected from a sheep of the twenty-second passage and an infective fly was isolated. It afterwards bit and infected eleven rats and they lived for 15, 15, 16, 17, 18, 18, 18, 20, 21, 21 and 25 days. A strain of *T. brucei* from Zululand appeared as an acute infection of such virulence for rats that the possibility of (CORSON, 1934) which seemed to have remained latent in a rat for several months, an accidental infection with *T. rhodesiense* was considered. *G. morsitans* were infected and an infective fly was isolated and fed on a susceptible volunteer who did not become infected and this fly afterwards infected eight rats. For this and other reasons an accidental infection was regarded as excluded. The virulence for rats was not diminished after passage through the fly.

Experiment.

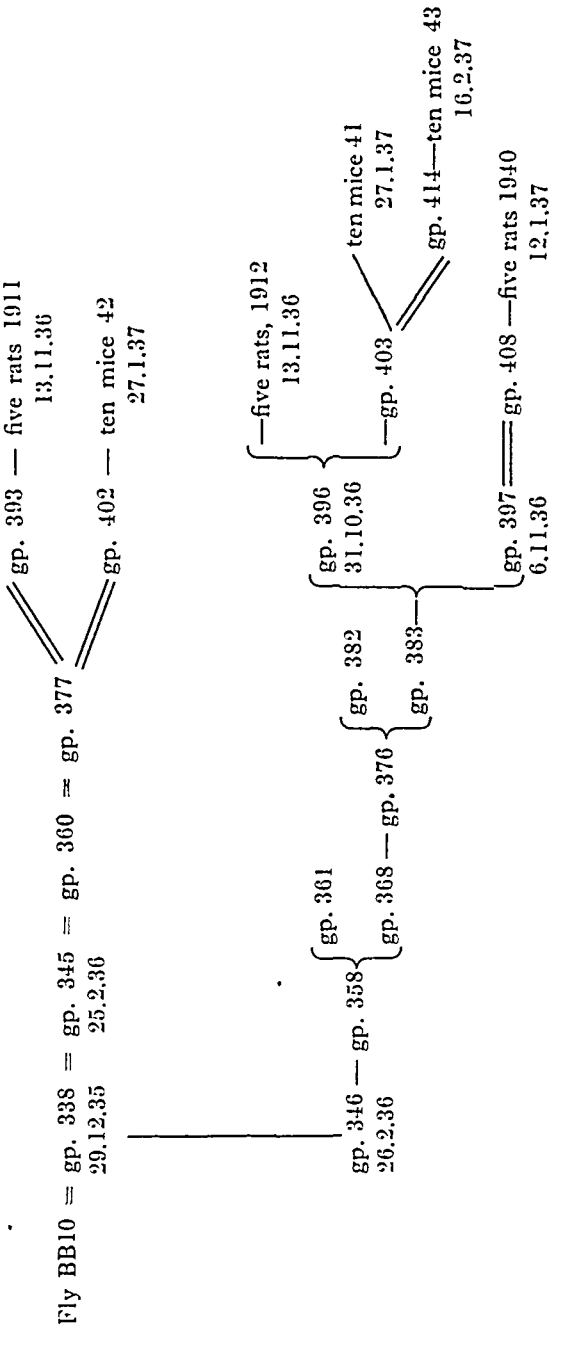
The following experiment was made with a strain of *T. rhodesiense* (*T. rhodesiense* Kahama) which was being maintained in sheep by cyclical transmission by *G. morsitans*. An isolated infective fly, Fly BB10, bit and infected Guineapig 338 on 29th December, 1935, and from this guineapig the infection was transmitted through two parallel series of guineapigs, in one series by *G. morsitans* and in the other series by inoculations with a syringe. The scheme is shown on p. 253; following a method used by Duke the passages by flies are shown by double lines, and those by inoculations with a syringe by single lines.

An attempt to transmit the strain by *G. morsitans* from Guineapig 396 failed but it succeeded from Guineapigs 397 and 403.

The following inoculations into rats and mice were made: five rats (No. 1911) from Guineapig 393 and ten mice (No. 42) from Guineapig 402, of the series of transmissions by flies; five rats (No. 1912) from Guineapig 396 and ten mice (No. 41) from Guineapig 403, of the series of transmissions by the syringe before the trypanosomes of this series were transmitted by flies; five rats (No. 1940) from Guineapig 408 and ten mice (No. 43) from Guineapig 414, these two guineapigs having been infected by flies from Guineapigs 397 and 403 respectively.

Until towards the end of 1936, when an epidemic occurred, there was evidence from the length of life of the guineapigs and from the numbers of trypanosomes in their blood, that the trypanosomes in the series infected by *G. morsitans* were less virulent for guineapigs than those of the series infected by the syringe. Guineapigs 338, 345, 360 and 377 lived for 107, 119, 132 and 150 days respectively, while Guineapigs 346, 358, 361, 368, 376, 382 and 383 lived for 124, 80, 84, 47, 62, 54 and 42 days respectively. This evidence was strengthened by comparing the duration of life, after inoculation, of Rats 1911 with that of Rats 1912, and of Mice 42 with that of Mice 41. In order to see whether cyclical passage had reduced this acquired virulence the duration of life of Rats 1940 was compared with that of Rats 1912 and that of Mice 43 with that of Mice 41. These figures are shown together with the incubation periods in the following table.

	Incubation Period.	Duration of Life in Days.
Rats 1911	7, 8, 8, 8, 8	57, 67, 82, 84, 90
„ 1912	5, 5, 5, 5, 6	22, 23, 25, 28, 61
„ 1940	5, 5, 5, 5, 6	13, 16, 18, 22, 32
Mice 42	4, 5, 5, 5, 5, 5, 5, 6, 6, 6	7, 12, 51, 61, 67, 72, 74, 76, 87, 96
„ 41	4, 4, 4, 4, 4, 4, 4, 4, 4, 4	9, 21, 28, 29, 29, 34, 39, 47, 48, 54
„ 43	4, 4, 4, 5, 5, 5, 5, 5, 5, 5	7, 8, 8, 22, 22, 22, 25, 29, 31, 32


Fly BB10 = gp. 338 = gp. 345 = gp. 360 = gp. 377
29.12.35 25.2.36
gp. 393 — five rats 1911
13.11.36
gp. 402 — ten mice 42
27.1.37
gp. 346 — gp. 358
26.2.36
gp. 361
gp. 368 — gp. 376
gp. 382
gp. 383
five rats, 1912
13.11.36
gp. 396
31.10.36
gp. 403
ten mice 41
27.1.37
gp. 414—ten mice 43
16.2.37
gp. 397
6.11.36
gp. 408 —five rats 1940
12.1.37

It appears that increased virulence for rats and mice developed in the series of guineapigs which were infected by inoculations with the syringe and that this increase was at least not diminished by passage through *G. morsitans*. The rats weighed from 60 to 80 grammes and the mice weighed about 20 grammes.

Summary and Comments.

An experiment was made to compare the virulence of a strain of *T. rhodesiense* in two series of guineapigs, the infection being maintained in one series by cyclical transmissions by *G. morsitans* and in the other series by inoculations with a syringe. It was found that the virulence had increased in the latter series and apparently not in the former. This was confirmed by subinoculations into rats and mice. It was also found that this increased virulence was not again decreased by passage through *G. morsitans*.

Very little is known of the factors concerned with the development of trypanosomes in tsetse flies, and of the influences of the tissues and body fluids of flies and vertebrates on the trypanosomes. Robertson (1929) in an account of experiments with *Bodo caudatus*, discussed the questions of selection, mutation and the heterogeneous composition of strains of trypanosomes. Plausible explanations of the variations in the results of transmission experiments could be based on such considerations. More work with infections by single trypanosomes seems to be needed.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 2. July, 1937.

MURINE TYPHUS IN EGYPT.

BY

D. RIDING, M.D., M.R.C.P.,

Deputy Director, Public Health Laboratories, Cairo, Egypt.

INTRODUCTION.

In a previous paper (RIDING, 1935) the writer gave his results, all negative, of an attempt to isolate a murine strain of the typhus virus from wild rats and mice in Egypt. The examination of wild rats has been continued, principally because PRIEST (1935) and others have recently reported sporadic cases clinically resembling endemic typhus among patients in Egypt.

During March and April, 1936, a further series of 54 wild rats captured in the village of Saft el-Melouk, Ityai el-Baroud Markaz, Behera Province, where cases of mild typhus were occurring, were examined in the Central Laboratories, Cairo. On 25th April, 1936, a strain of the murine typhus virus was isolated.

EXAMINATION OF THE WILD RATS.

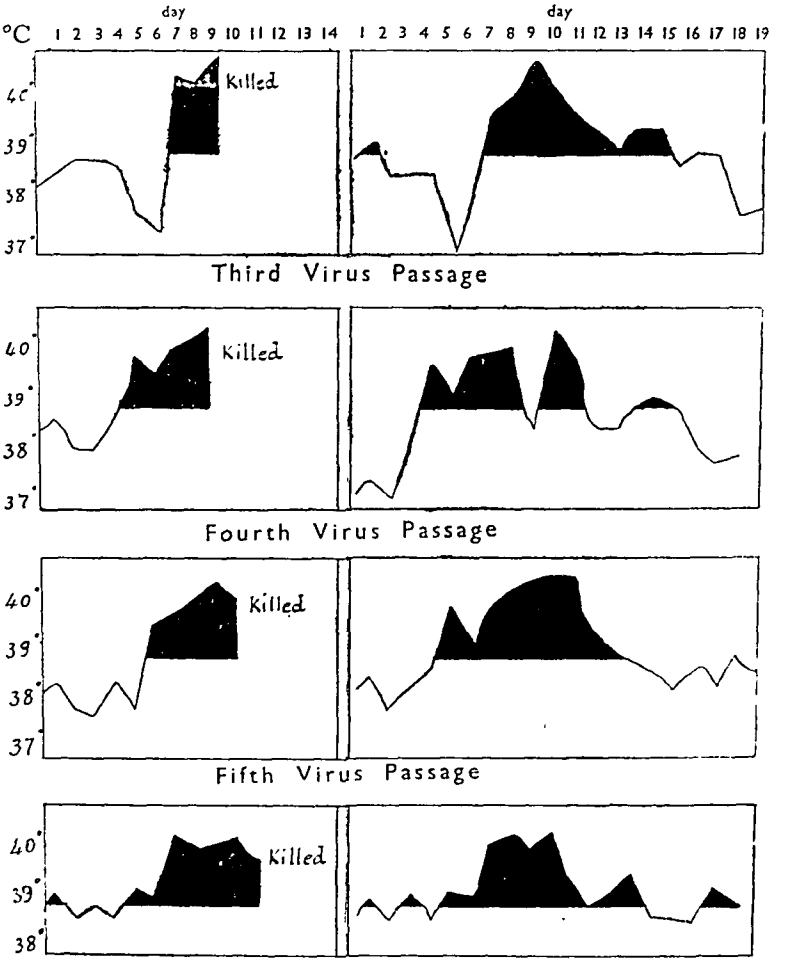
The rats consisted of: *Rattus rattus*, twenty-six; *Rattus norvegicus*, twenty-seven; *Acomys cahirinus*, one.

On arrival at the Central Laboratories, Cairo, the rats were killed with ether, heart blood was taken for blood culture and Weil-Felix test, the brain removed for guineapig inoculation, and a general postmortem examination carried out. No gross pathological lesions were noted in any of the rats. The heart blood cultures all remained sterile after 2 weeks incubation at 37° C. with the exception of one specimen from Rat 4 (*R. norvegicus*) in which a bacillus of the *Haemophilus* group was isolated.

Examination failed to reveal any infection of the rats with *Spirillum morsus muris* or *Leptospira icterohaemorrhagiae.*

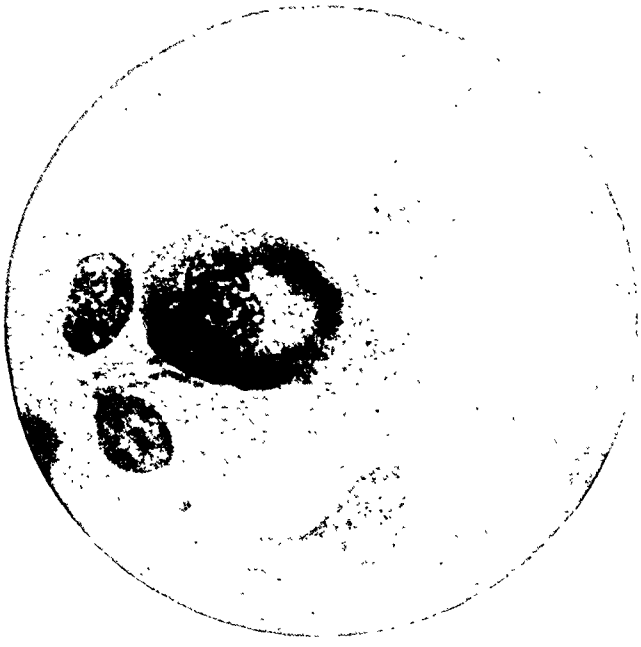
Agglutination tests were carried out with the sera of the rats against suspensions of *Proteus* OX19, OX2, and O Kingsbury, but in no case was a positive agglutination obtained in a dilution of 1 in 50 or over. It must be stated, however, that the Weil-Felix tests were carried out with *Bacillus proteus* suspensions prepared by the method of BRIDGES (1935) in order to obtain uniformity in the results. Unfortunately these concentrated alcoholized suspensions have only about 50 per cent. of the sensitiveness of living cultures.

The guineapig tests were carried out by pooling the brains of three or four rats, emulsifying these in normal saline solution, and injecting a quantity of the brain emulsion intraperitoneally into three guineapigs. By this means too many guineapigs were not sacrificed, and the occasional accidental death among the inoculated guineapigs did not destroy the experiment.



TEMPERATURE REACTION RECORDS.

Three guineapigs inoculated on 14.4.36 with the pooled brain emulsion of Rats 45, 46, 47 and 48 became infected with typhus. These rats were captured in two houses on 13.4.36 and it is interesting to note that a case of typhus was notified from one house on 24.1.36 and another case from the other house on 12.2.36.



ENDOTHELIAL CELL FROM TUNICA VAGINALIS OF A GUINEAPIG. × 1200.

8th day after infection with murine typhus, showing very many rickettsia some of which are escaping from the cell.

Photograph by Dr. Basili Farag.

To face page 257.

Experimental Typhus in the Guineapigs.

All three guineapigs inoculated intraperitoneally with the pooled brain emulsion of Rats 45, 46, 47 and 48 developed a well-marked febrile reaction, and a very definite Neill-Mooser scrotal reaction.

By using normal saline emulsions of the brains of infected guineapigs it has been possible to passage the virus twelve times up to date, between 26.4.36 and 15.8.36. Two guineapigs were used for each virus passage, one to supply the infected brain for passage, and the other to act as a control of the temperature and scrotal reactions.

The chart shows the temperature reaction records for the second, third, fourth and fifth virus passage. It will be noted that there is a well-marked rise of temperature after an incubation period of 5 to 7 days, and a period of fever of the saddle-back type of about 5 to 6 days.

The scrotal reaction was a very constant phenomenon in all the infected guineapigs, consisting of a very characteristic reddening and swelling of the scrotum appearing about the 5th day and persisting till the 12th day after inoculation. Dissection of the scrotum between the 8th and 12th day after inoculation showed marked congestion and fibrino-purulent exudation of the parietal portion of the tunica vaginalis, congestion only of the visceral portion of the tunica vaginalis, and great oedema of and occasional haemorrhage into the head of the epididymis.

In the guineapigs inoculated intraperitoneally with the brain emulsion from Rats 45, 46, 47 and 48 rickettsia were readily found in Giemsa-stained smears of the tunica vaginalis, but in all the passage guineapigs great difficulty has been experienced in finding them and then only occasionally. (See Plate.)

Tissue Cultures of the Rickettsia.

As rickettsia were only occasionally found in very small numbers in smears of the tunica vaginalis of the passage guineapigs an attempt was made to cultivate them in tissue culture.

The method of cultivation recommended by Aschner and Kligler (1936) was closely followed with the exception that glass stoppered Erlenmeyer flasks of 50 c.c. capacity were used to hold the Tyrode-guineapig serum solution instead of flasks of 25 c.c. capacity. After inoculation with the tissue the flasks were carefully stoppered, sealed with paraffin wax, and incubated at 35° C. instead of 30° C.

Successful cultures were obtained in 80 per cent. of the flasks after 8 to 10 days' incubation.

Experimental Typhus in Rabbits.

Rabbits inoculated intraperitoneally with either brain emulsion or saline washings of the tunica vaginalis of infected guineapigs although apparently ill

showed no definite febrile reaction. The serum of these rabbits gave an agglutination of a *Proteus* X19 O suspension obtained from the Standards Laboratory of the Medical Research Council (Oxford) and of a live OX 19 suspension in a dilution of 1 in 125 on the 14th day after inoculation. The titre of the serum did not rise by the 21st or 28th day, but in most cases was less on these days. No agglutination in a titre of 1 in 50 or over was present prior to inoculation.

Experimental Typhus in White Rats.

The intraperitoneal injection of white rats with either brain emulsion or scrotal washings from infected guineapigs failed to produce any temperature reaction, tunica vaginalis involvement, or production of serum agglutinins for *B. proteus* OX 19 in these animals. Actual infection of the white rats was proved however by the inoculation of their brain emulsion into guineapigs.

Summary.

1. A strain of the murine typhus virus has been isolated from wild rats captured in an Egyptian village.

2. The virus has been passaged twelve times up to date through guineapigs, and has produced consistent febrile and Neill-Mooser scrotal reactions in these animals.

3. Rickettsia have been grown from the tunica vaginalis of infected guineapigs in tissue cultures.

4. Rabbits inoculated with the virus have produced serum agglutinins for *Bacillus proteus* OX19.

5. Inoculation of white rats with the virus failed to produce any detectable effect, although actual infection was proved by inoculating rat brain emulsion into guineapigs.

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CORRESPONDENCE.

BEJEL.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

With reference to the paper by my friend, ELLIS H. HUDSON, on " Bejel—the endemic syphilis of the Euphrates Arab,"* may I contribute a few remarks.

In Kuwait and its outlying desert, the word " bejel," so far as I have been able to determine, is unknown. Our Bedouin, however, use the word "belesh " in much the same way as the Euphrates Arabs use " bejel." They are not always sure of their own diagnosis and I am frequently asked to give my opinion as to whether a lesion or eruption is belesh or not. I had been in Kuwait some years before I made up my mind that when the Bedou spoke of belesh, he was using *the desert name for syphilis.* The word " belesh " is never used by the people of the city of Kuwait, who call the disease " farinji," as on the Euphrates.

Among the Bedouin the initial chancre is rare. As on the Euphrates, the disease usually begins in the mouth.

HUDSON states : " General adenopathy is characteristic of bejel." These adenopathies among the Bedouin attracted my attention from the very commencement of my residence in Kuwait. I was told that they were tubercular and inasmuch as tuberculosis is extremely prevalent in the city of Kuwait, I decided that tuberculosis in the desert expressed itself in adenopathy and bone disease, rather than in pulmonary manifestations, for pulmonary tuberculosis is rare in our desert. But many of these patients were not particularly ill. They came with greatly enlarged cervical glands and apparently nothing else and asked to be treated. Many of them said they had belesh.

It is about 17 years since I began to use bismuth in these cases and in the treatment of belesh generally, and I have never turned back. The results are very encouraging. It would be most instructive to do some careful research work on these adenopathies so that we could positively rule out, or rule in, tuberculosis. It is quite likely that the two diseases frequently exist together in the same patient, and it is possible too that bismuth is of value in the treatment of tuberculosis.

Of the efficacy of bismuth in the treatment of belesh there is no longer the least doubt. For the past 8 years we have been giving 6,000 to 8,000 injections yearly in the American Hospital at Kuwait, in the treatment of belesh and also in the ordinary course of our syphilitic work. The injections have become more than popular and the people frequently ask for them. Our preference in Kuwait is for one injection every 5 to 7 days, rather than a small injection every day, although in selected instances we do follow the latter method. It multiplies work very greatly for the staff to give daily injections. Our usual course for an

**Trans. R. Soc. trop. Med. & Hyg.* (1937), 31.

adult man is four to six injections of 2 grains at each injection—the course to be repeated if necessary, and if possible with the elusive Bedou. Our people have now come to realize that our standard course is four to six injections and on the whole give us fair allegiance. It is not suggested for a moment that all patients undergo the full course—all too many drop out after one or two injections, either because of severe after-effects, for one gets an occasional troublesome stomatitis; or because no apparent improvement resulted; or it was too inconvenient to come so far; or . . . or . . . or . . . However, in Arabia, as elsewhere, one has to strive for an ideal.

As to the best preparation of bismuth to use, I have long since decided that sobita (Howards), or sodium bismuth tartrate, is the preparation of choice for our kind of work. It is so cheap that the cost is negligible. When I first began using it in simple aqueous solution I was greatly troubled by the amount of pain which so frequently followed injection. We now give it in a solution of 50 per cent. glycerine in distilled water, sterilizing the solution in glazed porcelain casserole dishes. The use of glycerine very largely eliminates the problem of pain subsequent to the injection. The injections are always made into the gluteus muscle, unless there are special reasons for using the deltoid. The patient should always be advised to rest for 24 hours after the injection, if possible. It is a fact which we have noted again and again, that patients in the wards never have any pain worth mentioning following injection.

I am, etc.,

Kuwait, Arabia. C. S. G. MYLREA.

TRYPANOSOMES IN BAT AND MARMOT.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

While visiting Berkeley, California, I had the opportunity of finding bats, *Antrozous pallidus pacificus*, infected with haemoflagellates. Parasites were found by fresh blood examination in two out of eight bats collected in a barn at Pinole, Contra Costa County, California. The trypanosome forms are morphologically similar to those of *Schizotrypanum cruzi* and were scanty in the peripheral blood. The material was obtained through the courtesy of Dr. HAROLD KIRBY, Jnr., of the Department of Zoology, University of California.

As far as I know, trypanosomes of bats have not yet been reported in North America.

At Hamilton, Montana, while working at the Rocky Mountain Laboratory, a trypanosome resembling *Trypanosoma lewisi* was found in one out of four woodchucks (*Marmota flaviventris nosophora*) examined.

I am, etc.,

Instituto Oswaldo Cruz,
Rio de Janeiro, Brazil. EMMANUEL DIAS.

THE FRESHWATER BIOLOGICAL ASSOCIATION OF THE BRITISH EMPIRE.

The general purpose of the Freshwater Biological Association is to investigate any side of freshwater biology, pure and applied. The English Lake District has been selected because it is an area in which one may find a great number of types of water, large and small, still and running, distributed over a considerable range of altitude. Work began at the end of 1931 and has been concentrated upon Windermere and streams that run into it. The Association's general plan is to have a group of people working in co-ordination upon the chemistry and physics of the water, the living things which occur in it, and the changes which follow drought, floods, or the progress of the seasons. At the present moment the team consists of an entomologist, principally occupied in determining insect larvae, an algologist, an investigator on salmon fry, and a worker on the animal plankton and the chemistry of the water. A Director has been very recently appointed, and he hopes later to carry out investigations on fish, a subject on which he has special knowledge.

It is thought that such work as this has considerable value to the Royal Society of Tropical Medicine and Hygiene. Could we understand clearly why certain species of *Anopheles* breed in certain waters it would effect an economy in sanitary measures and we might be able to produce some small but critical alteration in the types of water. This idea is not by any means new, indeed it has already been fruitful of results up to a point. But it is difficult for the isolated worker in the tropics to tackle so wide a problem. Perhaps the Freshwater Biological Association, with a library and a group of men concentrating their attention on one area, may hope to explain some of the causes of the abundance of organisms ; in so doing they would make a substantial contribution towards our understanding of *Anopheles*. It will also be remembered but there are many creatures important in human parasitology which pass a part of their life history in water and may be assumed to be limited by the factors prevailing in the water.

The laboratory at Windermere is a meeting place which is frequently used by people who are not members of the Association's staff. It may be of service to some member of our Society, anxious to expand his knowledge, improve his technique, or use the library. Any society making a contribution of £5 per annum to the Association's funds is entitled to nominate a person who can make use of the laboratory for one month without paying a bench fee. The Royal Society of Tropical Medicine and Hygiene has therefore voted a subscription of £5 for the current year. Any Fellow wishing to avail himself of the facilities on Windermere should apply in the first instance to the Society at Manson House, 26, Portland Place, London, W.1.

ANNOUNCEMENTS.

LEGACY TO THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

Dr. A. S. BURGESS, formerly of the West African Medical Service, and a Fellow of the Society since 1913, died on 15th March, 1937, leaving estate of the gross value of £26,507, net personalty £24,112.

After a number of small legacies (totalling approximately £5,000) Dr. BURGESS left the estate on trust for three persons during their lives.

After the death of these three the whole residue is to be divided in equal shares between the Royal Society of Tropical Medicine and Hygiene and Caius College, Cambridge.

Though it may be many years before the Society receives this legacy, it is gratifying to know that the position of the Society is being consolidated in this way.

Gifts such as these go far to ensure the future usefulness of the Society, and it is hoped that others will follow Dr. BURGESS's example.

OFFICERS OF THE SOCIETY.

At the first meeting of the new Council, held at Manson House, on the 1st July, 1937, appointments were made as follows :—

Honorary Treasurer.

Dr. OSWALD MARRIOTT was re-elected Hon. Treasurer for the ensuing two years.

Honorary Secretaries.

Dr. C. M. WENYON and Dr. N. HAMILTON FAIRLEY were re-elected Hon. Secretaries for the ensuing two years.

Local Secretaries

Fifty-one Local Secretaries were re-elected for a further term of two years. The following new Local Secretaries were appointed, subject to their acceptance of the Council's invitation: Dr. H. JOCELYN SMYLY, *North China*; Dr. N. KAMCHORN, *Siam*; Dr. T. B. GILCHRIST, *Cape Province*; Lt.-Col. E. B. MARSH, *Egypt*; Dr. G. S. ESCOFFERY, *Jamaica*.

CORRIGENDUM.

Vol. **31**, No. 1, June, 1937, p. 7, line 2.
Babesia canis should read *Babesia felis*.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE.

VOL. XXXI. No. 3. NOVEMBER, 1937.

Proceedings of the
Opening Meeting of the Thirty-first Session
held at Manson House, 26, Portland Place, London, W.1,
on Thursday, 21st October, 1937, at 8.15 p.m.
Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.), *President*,
in the Chair.

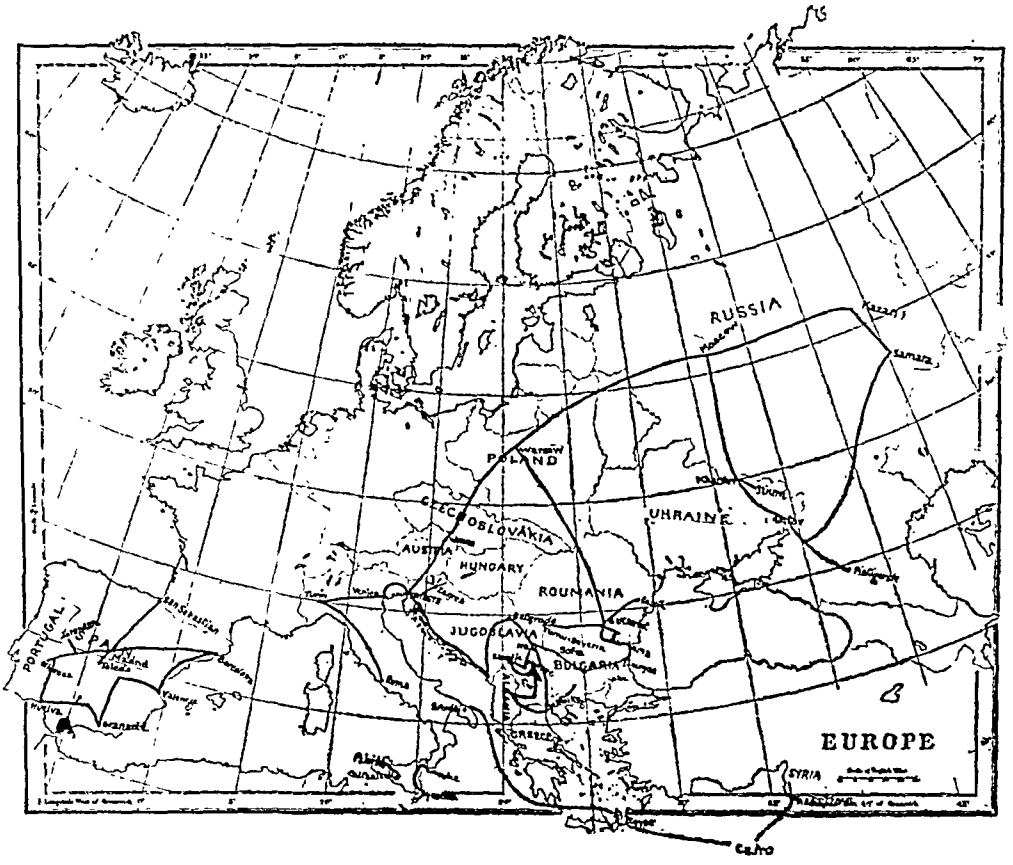
PRESIDENTIAL ADDRESS.

ADVANCES IN KNOWLEDGE OF MALARIA SINCE THE WAR.

BY
S. P. JAMES.

I think everyone interested in malaria will agree that the 20 years which have passed since the War were noteworthy first for the initiation and establishment of new and better arrangements for systematic research, second for some remarkable discoveries and additions to knowledge, third for a lively renewal of practical anti-malarial efforts in many parts of the world. My paper this evening is about these encouraging events which I believe have had the result of making the outlook for future success in the fight against malaria more hopeful than it has ever been. At any rate, it is more hopeful than it was 20 years ago when, as we all know, faith in the efficacy of every prophylactic and therapeutic measure had fallen almost to zero. Everyone who had actually taken part in efforts to deal with malaria in different parts of the

world during the War came home with the uncomfortable feeling that we knew much less about the disease than we thought we did, and that it might be quite a good plan to sink our pride and to begin again, in all humility and with greater respect and reverence, to try to fathom some of its mysteries. Opportunities for this new beginning soon became available by the spread of the disease in eastern and western Europe and by arrangements for what may be called the internationalization of malaria research and control. The idea of making malaria work a matter of international as well as of national concern had its origin in England in 1923 when various countries in Europe were beginning to recover from the effects of post-War epidemics and were trying to arrange for a continuous public health policy and a permanent medical and sanitary service for carrying it out. An initial difficulty in creating and developing the service was that the countries concerned were poverty-stricken and very backward in matters of medical assistance and public health arrangements. Doctors who had received a training in public health were few and far between and there was a great lack of subordinate personnel. Malaria was everywhere prevalent and severe and it was a question whether the public health policy should be based primarily on efforts to deal with this disease, or whether primary attention should be given to general medical and public health requirements which were equally of pressing importance. It was the old question whether malaria among poor illiterate people should be dealt with as an isolated problem separate from the general medical and public health problems of the country concerned. The situation was new to European experience and it was thought that useful advice about it might perhaps be given by a group of workers who had had experience of similar conditions in the tropics. In May, 1923, a proposal to this effect was presented to the Health Committee of the League of Nations which had been asked by several of the affected countries to advise them on the matter. The proposal was favourably received. The Health Committee appointed a small Malaria Sub-Committee, and later the Malaria Commission, which in 1924 undertook the collective enquiry that had been proposed. Seventeen members from eleven countries took part in the tour and in each country and district visited, representatives of the local administrative and medical staffs accompanied the Commission and took part in its discussions. Major NORMAN LOTHIAN, who had previously given promise of a brilliant career in the Royal Army Medical Corps, was Secretary to the Commission during this tour and again during the next collective tour which was made in Palestine and part of Asia Minor in 1925. On this latter tour, the Commission had the advantage of the participation of an expert from the United States, namely, the late Dr. SAMUEL DARLING, whose early work with General GORGAS during the construction of the Panama Canal is so well known. At the period of the tour in Palestine he was at the zenith of his powers as an expert adviser on anti-malarial work. Almost at the end of this tour an accident occurred in which he and LOTHIAN were killed. I had known and admired DARLING since we



STUDY TOURS OF THE MALARIA COMMISSION.

were together on the Panama Canal in 1911. His untimely death was a loss not only to the United States, but also to the world of research workers to which he belonged.

Another collective study tour undertaken by members of the Commission in 1925 was to Spain and another to Sicily; in later years there were tours to the United States, British India and other countries. I do not propose to say anything more about these collective study tours except to make the point that they were a new arrangement for the purpose of obtaining an impartial and authoritative pronouncement on measures for dealing with malaria in the light of existing knowledge and experience. They afforded an opportunity of ascertaining and comparing conditions in a number of countries. During the tours, observations made by individual members of the Commission were examined by other members and the results were discussed in full session by malariologists belonging to very varied schools of anti-malarial practice and opinion. These mutual discussions were the first occasion on which the collective thought of malariologists of different countries and different schools of teaching and practice were brought to bear on local malaria problems studied on the spot. They had the important result that individual views

became modified to the extent that sometimes, at any rate, the Commission as a whole were able to agree on what might be the wisest course to pursue in particular circumstances when due consideration was given to administrative and social and economic as well as to technical difficulties. I say sometimes because, as you probably know, agreement was not always reached. In that case it was usually decided that no general report on the tour should be published. The photograph reproduced on the opposite page was taken at a meeting of the Commission when thirty-five malariologists from about twenty different countries were called together to discuss a report which, in the end, it was agreed not to publish. Thanks to the meetings of this kind and to other arrangements made by the Commission for bringing malariologists of different countries into touch with one another, it is probable that there never was a time when workers on this subject knew each other so well.

One cannot speak of the internationalization of malaria work through the medium of the League's Commission without mentioning at the same time the International Health Division of the Rockefeller Foundation which during the last 15 years has financed the Commission's work. In addition to the international arrangements of the League, the Foundation has initiated and maintained from 1916 onwards an international arrangement of its own for systematic anti-malarial work in various countries. It is chiefly an arrangement by which particular methods for reducing the incidence of malarial infection, such as anti-larval work, screening and the treatment of carriers, are tried out experimentally on a small scale in the field. The arrangements are made in collaboration with Governments. They were begun in Arkansas, Mississippi and other Southern States, and gradually extended to localities in the Argentine, Porto Rico, Nicaragua, Brazil, and other countries in America. In 1924 and 1925 they were extended to Italy, Poland, Palestine, and the Philippine Islands. A map showing all the Rockefeller stations for studies on malaria in Europe is printed on page 267. Each of them is under the direction of a member of the staff of the Foundation's International Health Division. Some of them are called " stations for the demonstration of methods of control " but perhaps " research stations " would be a better name.

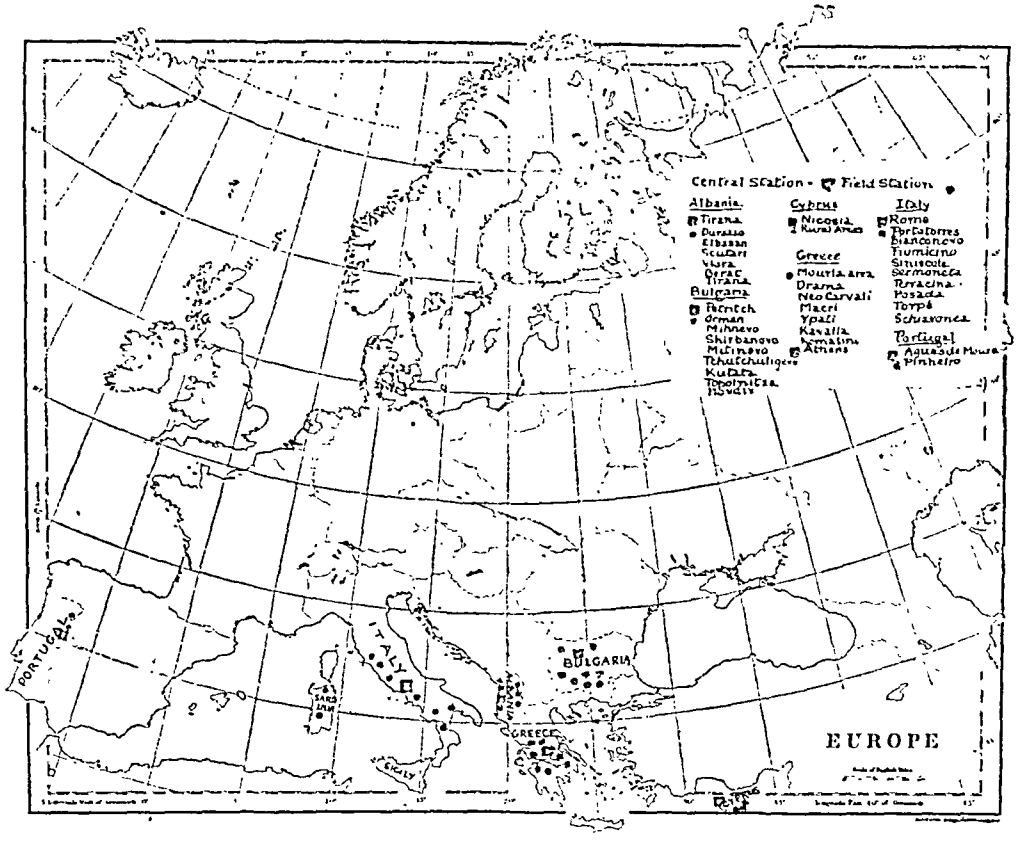
Altogether, during the 20 years ending with 1935, the Foundation spent on anti-malaria work, exclusive of the cost of field services, about two and a half million dollars, of which one and a half million was spent in foreign countries. I should add that the scope of the Foundation's malaria programme has not been limited to what are called in the United States " Malaria Control Demonstrations." There is hardly any item of malaria research, except perhaps chemotherapy, which has not received assistance directly or indirectly from its funds.

The grant of fellowships to enable medical graduates to become trained malariologists or to enable workers to see what is being done outside their own country is another beneficent activity of the Foundation. During the last few years nearly eighty fellowships and grants for these purposes have been given.

MALARIA COMMISSION OF THE HEALTH COMMITTEE OF THE LEAGUE OF NATIONS. JUNE 25th, 1928.



White. Markoff. Ciuca. Maxcy. Anigstein. Burnet. Taylor. Collins. Hackett. Rajchman. Strode. Swellengrebel. Evans. De Buen. Schilling. Boudreau. Marchoux. Boyd. Ascoli. Ferrell. Sfarcic. Ottolenghi. Labranca. Pittaluga. Missiroli. Mackenzie. Moutoussis. Kligler. Balfour. Lutrario. Schüffner. Nocht. James. Brumpt. Bonamico.
(Absent : Bailey. Cantacuzene. Christophers. Graham. Marzinowsky. Raynaud. Stanton. Wenyon.)



EUROPEAN MALARIA STATIONS.

Now let me mention another new arrangement which has been the means of greatly advancing our knowledge of malaria during the period under review. It began as a national arrangement which very soon became international. I refer to the establishment in mental hospitals, where malariatherapy is practised, of research laboratories charged with the duty of cultivating malaria parasites in mosquitoes and of inducing the malarial attacks by the bites of these insects instead of by direct blood inoculation from patient to patient. The arrangement opened up an entirely new field of clinical, epidemiological and therapeutic research on human malaria contracted in the natural way. The British Government, through its Ministry of Health in London, were the first to make an official arrangement for this work by the establishment of a malaria research laboratory at the Horton Mental Hospital, Epsom, in May, 1925. In March, 1926, by permission of the Ministry of Health, a report on the first results of the work was communicated to the League's Malaria Commission for presentation to the Health Committee and publication. Soon afterwards laboratories on the same model were established in several other countries, notably Italy, Holland, the United States and Roumania. In the United States there are two research centres of this kind, one maintained by the Rockefeller Foundation in Florida,

the other by the U.S. Public Health Service in Washington. Roumania also has two centres, one at Jassy, the other at Bucharest. There has always been active co-operation between the several centres named. It is maintained by personal visits and by the exchange of species and strains of the malaria parasites and mosquitoes with which the work is done. An interesting point connected with it was the finding that anopheles confined in the cages invented by Captain BARRAUD in India travel well by sea and air. Between 1931 and 1933, batches of anopheles were received alive at Horton from countries as far distant as British India, West Africa, Uganda and Trinidad, and on more frequent occasions from a number of countries in Europe.

New and better arrangements for research on other aspects of malaria were also a feature of the period under review. One of them was the renewal of anti-malarial chemotherapeutic research by the chemical industries in Germany and France and the creation by the British Government of an organization for systematic work in England. For various reasons, arrangements made in this country were on a small scale but since their initiation in 1925 some progress has been made, particularly with arrangements for testing new synthetic preparations and for studying the biological and physico-chemical principles underlying the action of quinine and synthetic drugs. There are special units for this side of the subject in Liverpool, London and Cambridge. Recently, the Medical Research Council, in consultation with the Department of Scientific and Industrial Research, have made plans for promoting chemotherapeutic investigation on a larger scale, for which the Government have provided an annual grant of £30,000. It is understood that the scheme includes the provision of a new Institute and that at any rate for the next year or more the income of the fund will be used to meet the cost of building. It will therefore be some time before expenditure on actual research work can be much increased.

Another Medical Research Council scheme is an arrangement by which young graduates who show an aptitude and desire for research are given grants and facilities for becoming specially trained as research workers in the tropics. It is understood that as qualified investigators become available under this scheme they will be eligible for permanent pensionable appointments for research work on malaria and other subjects of tropical medicine abroad and at home.

Lastly, I must not fail to mention arrangements made by particular societies and individuals. One example of each will suffice. Everyone will remember that the Royal Society of London, more than 35 years ago, followed up the discovery of the mosquito-cycle of the malaria parasite by sending research workers to Africa and India to study the new epidemiology of the disease and the life history and habits of the mosquitoes which transmit it. Last year the Society took a second step which everyone hopes will have equally important results. It was the decision to devote the major part of its Medical Research Fund to a scheme of malaria research consisting firstly of a study by modern experimental methods of the parasites and their relationship with human,

animal and insect hosts, secondly of an intensive study of the ecology of one or more of the species of anopheles which spread malaria in the tropics. As a recent example of arrangements made by individuals I may cite the establishment of the Dorothea Simmons Malaria Research Station in Greece where the remarkable work on blackwater fever described by Mr. FOY and Dr. HAMILTON FAIRLEY at our Society's meeting in June this year was done. The station was situated first in the Peloponnesus but for the last few years has been in Salonika. Unfortunately the donor's grant to this station will cease at the end of 1938. In view of the outstanding importance of its work on blackwater fever it is greatly to be hoped that a plan for keeping it going will be found.

II.

Now I must pass to the second part of my paper, that is to the discoveries and additions to knowledge made during the period under review. On this subject I think the outstanding feature of the advance of knowledge during the period was the discovery or invention of better ways of investigating the disease. After the War the uneasy feeling of ignorance which I mentioned at the beginning of my paper caused workers to look about for new avenues of approach to unsolved problems. Clinicians, epidemiologists and entomologists gave much thought to this matter. An early opportunity for new work was provided by the occurrence of cases of malaria in the families of soldiers who had returned to their homes. These occurrences were studied in detail with the object of ascertaining precisely the sequence of events which led to their onset and the manner in which they multiplied to constitute local epidemics. Attention was directed to the habits and behaviour of the insect vector in the adult stage, and to the circumstances in which it became infected and transmitted the infection in particular houses. In this way malaria began to be studied in individuals of selected families in their own homes as well as in the mass by random sampling in the village street, and anopheles began to be studied from the point of view of the behaviour of the adult female insect in these houses as well as from the point of view of breeding places of larvae in the general environment. These studies were the starting-point of work which led to the discovery of biological races of anopheles indistinguishable morphologically in the adult stage, but with different habits and therefore, sometimes, with a different rôle in the epidemiology of the disease which they transmitted. At first it was thought that the results of these household studies were of interest chiefly from the point of view of the epidemiology of malaria in Europe ; later it was found that their application in the tropics gave equally fruitful results, so it is now generally recognized that a "malaria survey," wherever it is carried out, must include on the one hand a clinical and parasitological study, over a considerable period, of selected individuals of particular age-groups in as many families as possible, and on the other a close study of the habits and behaviour of the insect vector

in the adult as well as in the larval stage. A good example of studies of the first type was the investigation in Nigeria made by the late Prof. J. G. THOMSON, whose recent untimely death we all so much deplore. Another is the investigation made by Dr. BAGSTER WILSON in Tanganyika. Results of the second type of study have led to quite a novel method of controlling malaria among uncivilized native races in South Africa.

Another avenue of approach indicative of the general desire to break new ground was investigation into the circumstances and factors to which the apparent disappearance of malaria from England, Denmark and Holland may have been due. These enquiries showed that the apparent disappearance had come about without any reduction of the insect vector concerned. Malaria is essentially a house or family disease and its spread is greatly facilitated by circumstances which bring gametocyte carriers, insect vectors and non-immune persons into close and continuous association It was found that in the countries named, this close association had been gradually broken in the course of years by progressive social, economic, educational, medical and public health improvements. Thus a way was opened which justified the application of other methods of dealing with malaria than those arising directly from the belief that because mosquitoes transmit the disease their elimination must be the object of chief concern and expenditure.

Other methods of investigation devised during the early post-War years were of a quite different kind. In 1924, the late Dr. ROEHL brought into application on a large scale at Elberfeld, in Germany, a method for seeking anti-malarial remedies by testing numerous quinine and quinoline derivatives to ascertain whether any of them were effective against the parasites of bird malaria. In 1925, as I have already mentioned, the practice of malariatherapy was applied systematically in England to the study of malaria itself as well as for its original purpose. Both these arrangements quickly led to great advances of knowledge on chemical and biological aspects of malaria and in particular to a change of thought and opinion on the aims and methods of anti-malarial therapeutics. On the chemical side an epoch-making advance was the discovery of plasmoquine and other synthetic compounds which promised to be effective anti-malarial agents. On the biological side additions to knowledge were no less important. The vexed question of the unicity or plurality of the human plasmodia was answered unequivocally. It is now accepted universally that the group comprises a number of species of which the morphological characters are quite distinct, and that in addition there are a number of varieties of each species with lesser morphological differences, such, for example, as those described for the indigenous type of *Plasmodium vivax* endemic in Holland as compared with those of the tropical type from Madagascar. In addition, it is accepted that within each morphological species and variety there are many strains having different biological properties ; they cannot be distinguished morphologically but can be separated by their clinical effects, immunological characters and their reaction

to anti-malarial drugs. Acquired tolerance or immunity as a result of repeated attacks was found to be specific not only as regards the various species of parasite concerned but also as regards particular strains. There were also notable advances of knowledge on the therapeutics of the disease. It was found that although quinine is one of the most remarkable drugs in the world it has several defects. In particular it does not prevent infection of the human host or the insect vector, and it does not prevent relapses. Its merits and defects in these and other respects were for the first time clearly ascertained and described; and when this was done it became possible to justify anti-malarial chemotherapy on scientific grounds. Its aim is not to supplant the one and only remedy that has been available for 300 years, but to supplement it with additional weapons for particular purposes.

Except the practice of malariatherapy no method of research contributed so much to the addition of knowledge during the period under review as did the study of avian malaria parasites and the extensive use of canaries and other birds harbouring these parasites for laboratory work. Everyone knows, of course, what we owe to researches on bird malaria: MACCALLUM'S discovery of the fertilization of the female gametocyte, Ross's working out of the mosquito cycle of the parasite, the early work on the curative action of quinine by KOPANARIS, the brothers SERGENT, GIEMSA and others, ROEHL'S drug-testing device which resulted in the discovery of plasmoquine, and the fundamental work of the TALIAFERROS on the mechanism of immunity. About 1931 the knowledge which had been gained on human plasmodia by their study in the practice of malariatherapy was applied in new studies of the known avian species. ROEHL'S method of chemotherapeutic test was supplemented by using canaries infected by mosquito bites instead of by direct-blood inoculation and tests were made on birds harbouring the parasite *Haemoproteus* of which the schizogonic cycle is passed in endothelial cells instead of in red blood corpuscles. Finally, new avian plasmodia were discovered and brought into use for experimental studies. One of them is the malaria parasite of the domestic fowl, which Professor BRUMPT found and described in 1935 and to which he gave the name *Plasmodium gallinaceum*, another is a malaria parasite of the Java sparrow which was apparently seen by ZIEMANN* in 1896 and by ANSCHUTZ in 1909 and by several workers since, but was nameless until 1935 when Professor BRUMPT called it *Plasmodium paddae*. It would be in accord with precedent if the intensive study that is now being devoted to bird malaria were to provide the solution to problems which, even with the facilities afforded by the practice of malariatherapy, cannot be investigated in the human subject. One of them is the completion of knowledge of the schizogonic cycle of plasmodia in their respective vertebrate hosts. The sporogonic cycle of these parasites in the mosquito has been known in every detail for nearly 40 years, but we know only

*ZIEMANN, H. (1937). *Zbl. Bakt.* I Abt. Orig., 140, 63.

a part of the story of their life in the vertebrate host. An important part of the story that we do not know is what happens to the parasite during the interval between the inoculation of sporozoites by the mosquito and the appearance of trophozoites in the red blood corpuscles. When Ross had worked out the cycle of the parasite in the mosquito the end of the story was quite naturally assumed to be that the sporozoites which this insect injects into the vertebrate host penetrate quickly into the red corpuscles of the circulating blood and become the trophozoites which commence the schizogonic cycle. Nearly everyone accepted this assumption and SCHAUDINN's observations in 1902 seemed to prove that the assumption was correct. GRASSI, it is true, tried without success to repeat SCHAUDINN's observations and always remained in doubt as to the destiny of the sporozoites, but to most workers it seemed against common sense to suppose that the parasite had to go through yet another cycle before entering red cells. So it was not until between 1925 and 1927 that the question again attracted attention. Its revival was due to the observation made by YORKE and MACFIE in Liverpool, and by ourselves at Horton, that the onset of a malarial attack intentionally induced by the bites of infective mosquitoes cannot be prevented by giving quinine during the incubation period. I showed an example of this observation at a meeting of our Society in 1931.* It is clear, in this example, that if the sporozoites which the thirty mosquitoes injected had immediately entered red blood corpuscles and had become trophozoites and schizonts the quinine would have killed them and no attack would have resulted. On the basis of these and similar experiments with plasmoquine the hypothesis was published in December, 1931, that what happens to sporozoites injected by the mosquito is that they are carried by the blood stream to the internal organs where they enter reticulo-endothelial cells and go through a cycle of development resulting in the production of merozoites which are able to enter red blood cells of the general circulation. Since then the problem of the destiny of sporozoites has been studied assiduously by many workers in different countries, and as a result it seems now that its solution may be close at hand. If so, it will provide an example of the manner in which advances of knowledge come about gradually as the result of observations made independently or collectively by many workers rather than as a result of isolated discoveries made by individuals. It would take too long to mention all the observations which have been made on this problem, and the names of those who made them, so I must limit myself to a brief statement of what seems to be the present position of knowledge on the matter. Three results of experimental work are of outstanding importance. The first is the observation that very shortly after the inoculation of sporozoites intravenously or intramuscularly, or by the bites of mosquitoes, the blood of the person or bird inoculated is not infective to other persons or birds, even when injected in large amounts, and that it remains non-infective for at least 2 or 3 days. This observation has been made repeatedly

* JAMES, S. P. (1931). *Trans. R. Soc. trop. Med. Hyg.*, 24, 477.

as regards the sporozoites of *P. vivax* and *P. falciparum*, and as regards several species of avian plasmodia. The second is the observation, which, of course, has been made only as regards bird malaria, that during the latter part of the period of non-infectivity of the blood other birds can be infected by inoculating a small portion of the spleen, the liver or the brain. Evidently these internal organs become infective earlier than the circulating blood. The conclusion drawn from these two observations is that sporozoites injected intravenously or by the mosquito are quickly cleared from the circulating blood and become lodged in the reticulo-endothelial system of the internal organs where they remain until they have developed to the stage which can become parasitic in the red cells of the circulating blood. The third outstanding result of experimental work is the discovery that some avian plasmodia have two schizogonic cycles of development in their vertebrate hosts: one occurs in reticulo-endothelial cells in the brain, spleen, liver, kidneys, lungs and bone marrow, the other in the red corpuscles of the general circulation. It is known that after infection by the bites of mosquitoes the cycle in the reticulo-endothelial cells precedes the cycle in red blood corpuscles because, as I have just mentioned, birds can be infected by inoculating them with a small portion of blood-free tissue from the spleen or liver or brain at a time when they cannot be infected even by inoculating a large quantity of blood. At the Laboratory Meeting of the Society which was held last March I had the pleasure of showing with Dr. TATE preparations of various stages of this endothelial cell cycle as it occurs in the parasite *Plasmodium gallinaceum* of the domestic fowl. Professor RAFFAELE, of Rome, had previously discovered stages of the same cycle in canaries infected with *P. elongatum* and *P. relictum*, and Drs. HUFF and BLOOM, of Chicago, had discovered what seemed to be the same stages in cells of the bone marrow of birds infected with *P. elongatum*. Dr. TATE at Cambridge, and Dr. KIKUTH at Elberfeld, have confirmed RAFFAELE's findings with regard to *P. relictum*, and Dr. KIKUTH has obtained the same results with *P. cathemerium*. Professor BRUMPT and others have confirmed the findings with regard to *P. gallinaceum*. It has not yet been proved that the stages seen in endothelial cells are actually stages of the growth and development of sporozoites taken up by these cells from the blood stream after their injection by the mosquito, but it is difficult to suggest any good reason against this view. It must be remembered, of course, that it is not yet justifiable to assume that the sporozoites of the human plasmodia have to undergo the same kind of preliminary growth and development in endothelial cells of the internal organs before they can become parasitic in red corpuscles of the general circulation, but if, ultimately, this should be found to be the case, we should be able to explain more satisfactorily than at present the failure of such remarkable anti-malarial drugs as quinine, plasmoquine and atebrin to act as true prophylactics, and to stop the occurrence of relapses. In the meantime it seems clear that the next step in anti-malarial chemotherapeutic research should be to search for a compound which will destroy avian plasmodia during their cycle in endothelial cells.

I have talked long on the advances of knowledge resulting from the study of bird malaria, but I must not omit to mention that during recent years notable additions to knowledge have also come from studies on malaria in monkeys. The systematic use of these animals for experimental work dates only from 1932, when NAPIER and CAMPBELL, and KNOWLES and DAS GUPTA in India described malaria parasites from the blood of naturally infected monkeys imported from Singapore. Since then several of these parasites have been the subject of intensive study particularly in relation to immunology, and one of them, *Plasmodium knowlesi*, is quite commonly used, especially in Roumania, for the practice of human malariatherapy. Most of the additions to knowledge have come from work done in British India by the late Colonel KNOWLES and his collaborators, and by SINTON and MULLIGAN. The results of a remarkable collaborative study by WILLIAM TALIAFERRO and W. H. MULLIGAN, of the histopathology of malaria, based chiefly on work done on *P. knowlesi* in India, have recently been published. During the last few years Sir RICKARD CHRISTOPHERS, who directs a special unit for research in malaria at the London School of Hygiene, has used *P. knowlesi* for an entirely new type of fundamental or basic research which is concerned with the physico-chemical principles underlying the mode of action of quinine and the synthetic anti-malarial compounds.

III.

In the third part of my paper I hoped to deal adequately with advances of knowledge on anti-malarial work in the field. But I find that for lack of time I must be brief. An outstanding event of the period was the bringing to light of malarious conditions in Europe comparable with those in very malarious parts of the tropics. They had always existed, but their rediscovery attracted much attention. Medical men, public health specialists and entomologists from various parts of the world came to study them and many specific anti-malarial campaigns were started. The Malaria Commission of the League of Nations played a large part in bringing the conditions to light, and the International Health Board of the Rockefeller Foundation are doing a great work in assisting the Governments of affected countries to fight the disease. You have already seen the map of Europe showing thirty-nine areas in each of which a special anti-malarial campaign is in progress under the direction of a member of the Foundation's staff. Perhaps when you looked at the map you wondered why so much anti-malarial work is being done in Europe when so much has been left undone in the tropics. This is one of the problems of malaria that still remain unsolved. You may have wondered, too, how all this good work is to be continued by the Governments of the countries concerned when the experts from abroad who now direct it have departed. This raises the question of the *creation of permanent official anti-malarial organizations* which is the most important matter with which any attempt to review recent advances in anti-

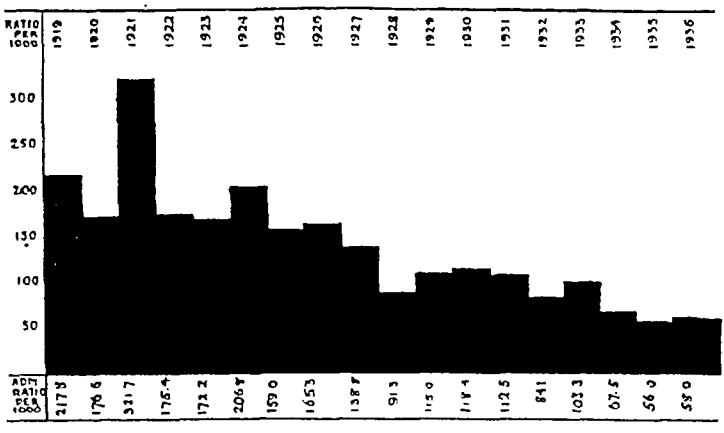
malarial field work should be concerned. The Malaria Commission, in their general report on the principles and methods of anti-malarial measures in Europe, placed the creation and development of a permanent service first among their recommendations. They advised the establishment at the headquarters of the Governments concerned of a central official organization similar to that which is usual for tuberculosis and other social diseases. They suggested that the organization should be a subsection of the Central Health Department and that its chief duties should be to advise the Governments on anti-malarial policy and measures, to arrange for malaria research, to arrange for training medical officers and subordinate personnel in malariology, to conduct malaria surveys and to give advice and assistance in the conduct of local measures. It is understood, or at any rate it is hoped, that the anti-malarial activities in Europe, in which the League of Nations and the Rockefeller Foundation are taking so great a part, are part of a programme leading to that end. But, as I said in the beginning of my paper, a chief post-War difficulty in European countries most affected by malaria was the absence of a health service of any kind and in many rural areas the absence of effective arrangements even for treatment of the sick. To create these services medical men and subordinate personnel had to be enlisted and trained and it was therefore a wise plan to begin at the periphery by using malaria as a starting-off point. But the goal aimed at is the development of a general rural medical and health service which provides for adequate medical attention in sickness of all kinds, for improvements in housing, water supply, conservancy and general welfare, and for the organization of educational campaigns designed to get the people to understand and to co-operate in the measures taken for their benefit. When this general service has been organized, arrangements for dealing with malaria will take their rightful place in it in accordance with the relative importance of this particular disease in comparison with the importance of other diseases and conditions affecting the public health. In some other countries, where medical and public health arrangements are more advanced, it has been possible to begin the organization of a special anti-malarial service at the centre rather than at the periphery. British India is a notable example of a country in which this has been done, and it is a country, too, in which definite advances in practical anti-malarial arrangements were made during the period under review. A few years after the War an official central organization called the Malaria Survey of India was inaugurated and financed by the Central Government to the extent of 2 lakhs of rupees a year. Its primary function was to conduct research into all branches of malariology, to act as an information and advisory bureau, to prepare and issue bulletins for the use of executive malariologists and public health officers, to train medical graduates in laboratory and field malaria work, to maintain a reference library, to identify specimens sent for opinion and to undertake special investigations in various parts of India for the purpose of carrying out measures of control. The wide field of research

undertaken by the Survey during the last 10 years is indicated by the numerous original articles published in the *Records of the Malaria Survey of India* which is edited by the Director. Many major field enquiries were conducted by research workers of the organization during the period as well as an investigation lasting from 1923 to 1931 into the therapeutics of malaria with reference to the efficacy of the cinchona alkaloids in comparison with quinine. Apart from this official organization maintained by the Central Government the different Provincial Governments have their own organizations, acting partly through institutes such as the Institute of Hygiene and the School of Tropical Medicine at Calcutta, the King Institute at Madras and the Assam Institute at Shillong, partly through the Public Health Departments, most of which maintain special malaria officers with assistants and field laboratories. In addition there are many local organizations for research and anti-malarial measures, such as the branches of the Ross Institute, which are chiefly occupied with industrial malaria in tea gardens, the special organizations for railways in Bengal and for reclamation and construction works in various parts of the country, the organizations in Indian native states, Mysore, Travancore, Patiala, etc., and the special anti-malaria staffs maintained by large cities, such as Bombay, Calcutta and Delhi. The campaign in the Delhi urban district is being conducted over an area of 55 square miles and is under the direct control of the Malaria Survey of India. The cost of the permanent works included in the first year's programme of measures was 14·75 lakhs of rupees and the cost of the temporary measures Rs. 75,000.

Now I must leave the question of organization and turn to advances in particular anti-malarial methods. First about treatment, everyone knows that some of the most striking advances of knowledge on malaria since the War were on the therapeutics of the disease. I need not say much about them because they were described fully in a discussion on synthetic anti-malarial remedies and quinine which was held at this Society in 1932, and in a general report published by the Malaria Commission of the League of Nations in 1933. A second general report on therapeutics by the Commission is now in the Press. In the work that has been done two quite different aims have been consistently kept in view. The first is to provide a remedy that is so abundant and can be obtained so cheaply that it can be made readily available to the whole population of malarious countries. The second is to find preparations which, regardless of their cost, will be effective for purposes for which quinine is known to fail. On the first aim the chief advance was the finding that for the treatment of the great majority of cases of malaria occurring among the indigenous inhabitants of malarious regions quinine has little or no practical advantages over the mixture of alkaloids known in India as cinchona febrifuge and in Europe as totaquina. Both are cheaper products than quinine. On the second aim an epoch-making advance was the discovery of plasmoquine, atebrin and various other synthetic anti-malarial remedies. The Malaria Commission's second general report

describes the results of clinical trials on a large scale made in various countries between 1933 and 1936 with the object of comparing the efficacy of these synthetic preparations with that of quinine. I think the results confirm most of the conclusions stated in the Commission's previous report and provide valuable further information on some questions that could not be answered definitely in 1933. One of them was whether a short course of plasmoquine given in doses of 0·02 gramme twice a week after cure of the attack by quinine reduces relapses or not. This is now answered definitely in the affirmative. It will be remembered that this practice was introduced some years ago in a standard plan of treatment adopted for the British Army in India, and that the remarkable decline of admissions to hospital illustrated in the graph below

A GRAPH SHOWING THE ANNUAL INCIDENCE OF MALARIA (NUMBER OF ADMISSIONS PER 1000 OF STRENGTH) AMONGST BRITISH TROOPS (OTHER RANKS) IN INDIA FROM 1919 TO 1936



was attributed largely to it. I wish I could have referred to other conclusions stated in this valuable report, but the Commission has taken such care to make reservations with regard to them that I have not found it possible to summarize them briefly.

Knowledge and experience in anti-mosquito work and methods advanced considerably during the period and some noteworthy successes in reducing the prevalence of particular species of anopheles were reported. Many inventions for killing larvae, for preventing anopheles from breeding in certain waters and for killing the adult insects were made, but most is expected from the new knowledge on what are called biological or natural methods of control which has been a feature of research in recent years. The successful employment of these

measures in several campaigns are described enthusiastically in Dr. L. HACKETT's admirable book, *Malaria in Europe*, which I am sure all of you h read. Increased attention is also being given to so-called " species-sanitatio although like most methods its practical application has been found to difficult, particularly in the United States where the idea (as first put forw by CARTER and DARLING for dealing with *Anopheles quadrimaculatus*) did get a favourable reception. This is chiefly because in the United States a malarial practice is complicated by the fact that although malaria as a dise is being dealt with sufficiently by the personal and household measures wh the people themselves are already taking, or can easily be persuaded to ta these measures do not ameliorate the serious prevalence of mosquitoes wh cause so much discomfort and annoyance in most localities, both urban a rural. To be rid of mosquitoes of all kinds is what the people wa No public health department in the United States can afford to neg this desire, which is sometimes expressed in forcible language and is governing factor in financial appropriations towards " malaria contro This makes it difficult to do selective control work on the different spec On this subject of the attitude of the inhabitants of a country towards application of particular methods an interesting discovery made in recent ye is that, in some parts of the world, killing adult mosquitoes by the use insecticidal sprays in houses appeals more to the inhabitants than anti-lar work. The practice has been applied systematically on a large scale native huts in Natal and Zululand, and has been reported on as being willin and enthusiastically received, as costing only about a third of the cost of ar larval work and as being more effective in controlling malaria. The sa method is being tried in campaigns in India.

On the whole, it must be admitted, I think, that existing anti-mosqu measures are still very crude and that no striking advance can be expect until we know more of the life history, habits and behaviour of these " pes ferous insects." I have already mentioned that the Royal Society is n interesting itself in this most important subject.

I had hoped to say a few words about advances in other anti-malar methods, particularly reclamation of land, bonification, housing, screening a propaganda, but I must end. I am afraid that the account which I have giv you of what has been done about malaria since the War is incomplete ar imperfect, but I hope that men of reason and goodwill may find in it some cau for a brighter outlook than seemed possible twenty years ago.

The Meeting was preceded at 7.45 p.m. by a Demonstration, arranged b Colonel JAMES, of microscope preparations of the development of the followi avian plasmodia in endothelial cells: *P. elongatum*, *P. relictum*, *P. cathemeriu P. gallinaceum*. The exhibit included preparations kindly sent by Profess RAFFAELE (Rome), Dr. HUFF (Chicago) and Dr. KIKUTH (Elberfeld).

VOTE OF THANKS.

Sir Rickard Christophers: Mr. PRESIDENT, ladies and gentlemen, it is not customary, following the PRESIDENT's address, to have a discussion on the lines that we hold on our ordinary meeting nights, but in proposing a vote of thanks to our PRESIDENT for the very interesting and instructive address which he has given us to-night, some comment on the subject of that address is, I think, in order.

This, I think, is the first occasion on which a Presidential Address at this Society has dealt with malaria in general. I think those of us who have just listened to our PRESIDENT will have been greatly impressed with the complexity of these new developments in regard to malaria. It is not without reason, I think, that malaria is the only disease which has abrogated to itself a complete science. Though the words "malariology" and "malariologists" may be very barbaric, they have evidently come to stay. One does not hear of "pestology" or "cholerology" for plague or cholera, or—however important hookworm disease may be considered—of "ancylostomatology." But malariologists are well known, in fact, malariologists seem to increase and multiply in a most extraordinary manner. I should not be surprised if there are twenty or thirty malariologists in this room to-night.

Those of us who are malariologists will have been able to appreciate, perhaps more than others, the amusing description which our PRESIDENT has given us of those collective tours by all the high lights of malaria—the international brass-hats, one might call them—wandering about Europe. I have heard the suggestion made by someone who knew a great deal about malariologists that a very interesting book might be written concerning these tours. There is no doubt that these meetings of malariologists from all over the world have produced a most profound effect, as the PRESIDENT well indicated this evening, on the study of malaria. The indirect effect of this exchange of views alone is very great.

To many of us, however, it was probably the second part of our PRESIDENT's paper which was the most interesting, namely, that in which he gave a resumé of the recent developments emerging from a study of this disease. With Ross's discovery of the mosquito cycle one would have thought that the whole subject had been permanently closed. This may be largely so as regards the main facts in aetiology, but otherwise it has been quite the reverse. In the last 20 years especially so many new channels have been opened up that the subject has become almost hydra-headed. What we have not been told to-night is the very large part which our PRESIDENT himself has taken in almost all these recent lines of development. Whether it has had to do with immunity in malaria or the habits of the mosquito, or with new work on relapses and many other interesting points, Colonel JAMES's name, perhaps more than that of any other

single individual, comes forward. He has been the influence at the back of a great amount of the work which has been done on these lines.

It gave me great personal pleasure to hear our PRESIDENT refer to the question of the organization of malaria work in India, and especially what he said about the Malaria Survey of India. I think this organization can well be proud of the high level of work which it has put out, and of some of the unique lines it has followed. It is practically the originator of the idea of the malaria surveys, now recognized to be so important as the basis of all active work against malaria. The functions of the survey were described to us by our PRESIDENT. Perhaps the most important function of all is that it forms a kind of malaria brain for India. In this respect, the position it holds as adviser to the Goverment and in the control of research in India is a very desirable and effective one. Malaya has a somewhat similar organization, though on rather different lines. It, too, has turned out magnificent work.

It gives me great pleasure to propose a vote of thanks to our PRESIDENT for his delightful address to-night, and I should like to include also our thanks for the very fine demonstration of specimens he has given us to-night.

Dr. C. M. Wenyon said he had great pleasure in seconding the vote of thanks proposed by Sir RICKARD CHRISTOPHERS.

The vote of thanks was carried, and the PRESIDENT thanked the meeting for thus expressing their appreciation.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

COMMUNICATIONS.

SELLAR FEVER.

BY

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1. DEFINITION.

A mild fever showing a "saddle-back" temperature chart and lasting in the typical form for 5 days, but which may have a duration of any period from 12 hours to 6 or more days. It is accompanied by frontal headache, congestion of the throat, and slow pulse, and there is a special liability to relapse.

2. INVESTIGATION.

The investigation of this disease has been carried out amongst Indian troops, as opportunity served, for many years in the following places in India: Risalpur, Malakand, Chakdara, Abbottabad and Razmak in the North-West Frontier Province, and Ambala and Sialkot in the Punjab. The principal investigations took place in Razmak in 1932, Ambala in 1933 and in Sialkot from 1934 to 1937.

Razmak is situated in lat. 33° N. at a height of 6,500 feet above sea level. In summer the maximum shade temperature does not reach 90° F., and there is a frequent rainfall; in winter there is snow. Abbottabad, lat. 33° N., height 4,000 feet, is a semi-hill station with a maximum summer shade temperature of 97° F. and snowfall in winter, while Ambala, lat. 30° N., height 900 feet, and Sialkot, lat. 35° N., height 800 feet, experience the climatic conditions of the plains of northern India.

More than 1,000 cases have been observed personally. There has been no opportunity of observation amongst British troops, nor in other parts of India.

3. INCIDENCE.

In Sialkot, during the year 1936, out of a total number of 750 admissions (373 medical, 377 surgical) of Indian combatants to the Indian Military Hospital, no less than 126 were on account of this disease, while, in addition, 398 more received treatment either as outpatients or in the detention ward, bringing the total number of cases to 524 for the year. As the average daily strength of combatants was 1,976, this gives an incidence of 265·18 per thousand.

The total number of days of duty lost was 2,492; that is, more than one quarter of the Indian troops in the station each lost, on the average, over $4\frac{1}{2}$ days' duty during the year.

Since the admissions of combatants to hospital in Sialkot for this disease during the years 1934 and 1935 (with approximately the same daily average strength) were 145 and 91 respectively the incidence in 1936 may be considered as normal.

4. Description.

(*a*) Incubation period.

A careful study of the incidence in various units has failed to show any regular interval between cases. Apart from sporadic cases, the interval between cases in a barrack room varies between 5 and 20 days. This is not sufficiently constant to be regarded as the incubation period.

(*b*) Onset.

Occasionally there is a prodromal sense of malaise or fever for a few hours. The onset is sudden, perhaps accompanied by shivering. Patients can state precisely the time at which they were taken ill. Frontal headache, frequently severe, and a sense of fever are invariably present. Sore throat is complained of in 75 per cent. of cases, and there may be backache, general pains, or pain in the chest or abdomen. Severity or mildness of the onset bears no relation to the duration of the attack.

The time of onset is frequently at night; 40 per cent. of cases commence between 6 p.m. and 6 a.m.

Table I.

Time of onset.

Time in hours.	Cases.	Percentage.
00.00 to 03.00	16	8·6
03.00 to 06.00	10	5·3
06.00 to 09.00	26	14·0
09.00 to 12.00	25	13·4
12.00 to 15.00	26	14·0
15.00 to 18.00	34	18·3
18.00 to 21.00	32	17·2
21.00 to 24.00	17	9·2
Totals	186	100·0

(*c*) CLINICAL COURSE.

Typically, the duration of the disease is 5 days, but many variations are encountered. The course may be cut short at any time from 12 hours onwards, and occasionally may last beyond 5 days. It may be prolonged by a relapse or relapses.

Patients often do not report till the second or third day of the disease. Many of the cases which appear to last for more than 5 days come under this heading and their history must be regarded as doubtful. Such cases are, however, shown in the following tables in accordance with the history given.

TABLE II.

DURATION OF FEVER IN 411 CONSECUTIVE CASES OF INDIAN COMBATANTS AND FOLLOWERS ADMITTED TO THE INDIAN MILITARY HOSPITAL, SIALKOT IN 1934-35-36.

Duration of Fever.	1934.		1935.		1936.		Total.	Percentage.
	Comb.	Foll.	Comb.	Foll.	Comb.	Foll.		
1 day	1	—	5	—	12	1	19	4·62
2 days	23	—	22	3	22	5	75	18·25
3 „	38	3	18	2	26	—	87	21·17
4 „	31	6	15	8	17	2	79	19·22
5 „	30	1	16	5	27	2	81	19·71
6 „	4	—	3	1	3	2	13	3·16
7 „	1	—	—	1	1	—	3	0·73
8 „	—	—	1	—	—	—	1	0·24
Relapses	14	1	9	4	18	7	53	12·90
Totals	142	11	89	24	126	19	411	100·00

The rise in temperature reaction may reach as high as 104° F., or may, on rare occasions, be so slight as merely to be half a degree above the individual's normal, but without rising above 98·4° F.; all intermediate severities are met with. There is never any toxaemia; the general appearance of the patient gives no cause for anxiety. A striking feature of the disease is the mildness of the discomfort, except perhaps for the headache, even when the temperature is high and prolonged by relapses. Three-quarters of the cases reporting sick

do not require admission to hospital. Many, who have only a history of fever the previous day, are treated as out-patients and others, who have only slight fever for a short time, are treated with one or two nights in the detention ward.

Convalescence is rapid and nearly all cases are fit to return to full duty within a few days of the return to a normal temperature. No after-effects are left.

Temperature.—A striking characteristic of the disease is the saddleback appearance of the temperature chart. This is caused by a distinct but temporary

TABLE III.

DURATION OF FEVER IN ALL COMBATANT CASES REPORTING SICK AT THE INDIAN MILITARY HOSPITAL SIALKOT IN 1936.

Duration of Fever.	Outpatients.	Detained.	Admitted.	Total.	Percentage.
1 day ...	164*	118	12	294	73·09
2 days ...	—	67	22	89	383
3 „ ...	—	33	26	59	11·26
4 „ ...	—	4	17	21	4·01
5 „ ...	—	7	27	34	6·49
6 „ ...	—	—	3	3	0·57
7 „ ...	—	—	1	1	0·19
Relapses ...	—	5	18	23	4·39
Totals ...	164	234	126	524	100·00

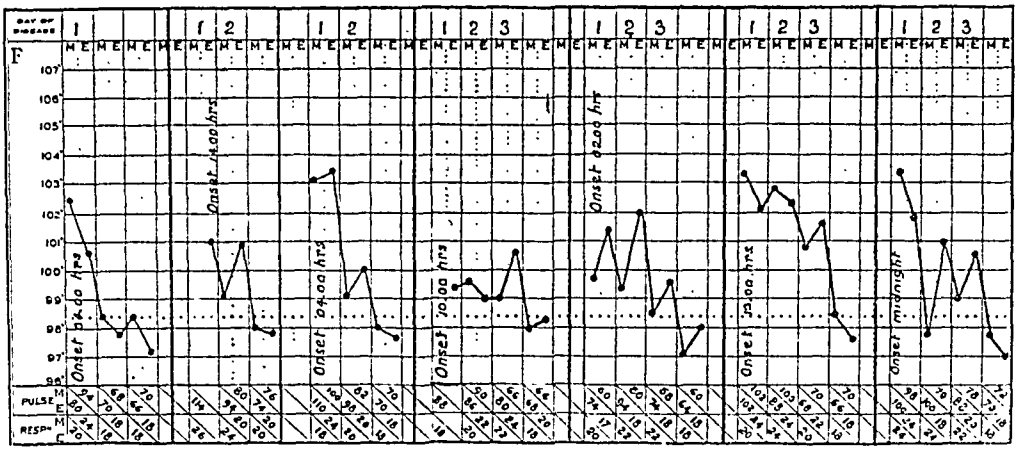
*Accurate details of the duration of disease of those treated as outpatients are not available. In the majority of cases it only lasts for 1 day.

drop in the earlier part, perhaps to normal limits, which divides the course into two phases.

This remission usually commences on either the second or third morning, but may commence as early as the first evening (when the onset is during the previous night), and lasts from 12 hours to 2½ days.

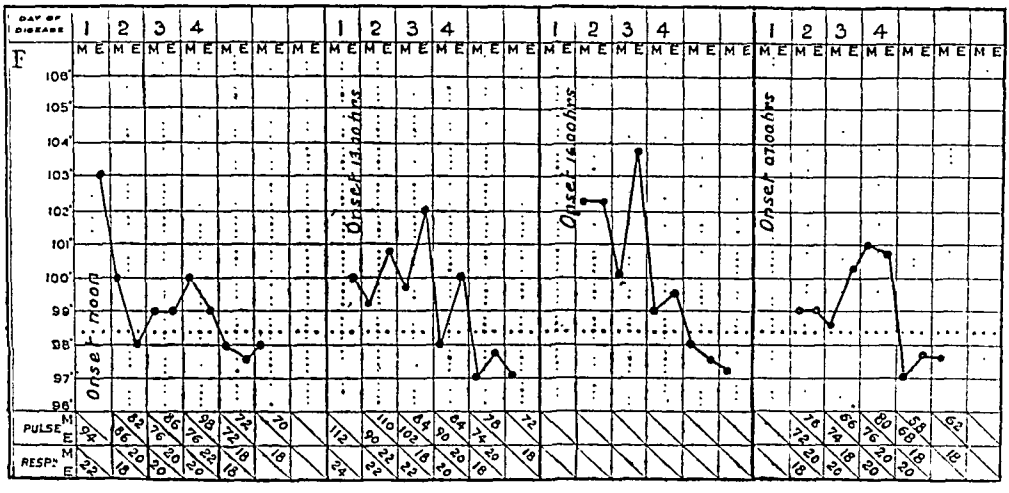
Table IV shows the time of commencement and the duration of the remission in 178 consecutive cases of 4 or more days' duration. No useful information can be obtained from a study of cases of shorter duration. In

I. Sepoy P. M. II. Recruit K. M. III. Recruit P. S. IV. Sowar C. S. V. Sapper T. J. VI. Sepoy S. R. VII. Sowar S. S.



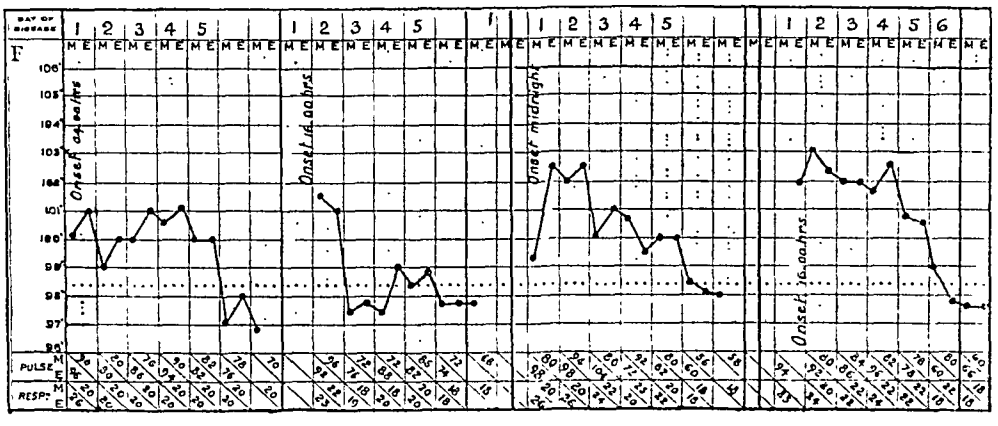
CASES OF ONE, TWO AND THREE DAYS DURATION.

VIII. W/C K. M. IX. Naik P. N. X. Sepoy K. N. XI. Recruit A. S.



CASES OF FOUR DAYS DURATION.

XII. Sowar A. K. XIII. Recruit S. S. XIV. Recruit G. K. XV. Sepoy A. S.



CASES OF FIVE AND SIX DAYS DURATION,

these the remission can often be observed, but the course of the disease is not long enough to show the typical saddle-back. Cases of 3 days' duration occur with a remission on the second day which may give rise to a suspicion of malaria.

It is also to be noted that only cases in which the remission is *obvious* have been included. In many of the cases shown as having no obvious remission, indications may be seen, *e.g.* (*i*) A failure of the normal evening rise on the second evening, or (*ii*) An indication of the onset of the second phase by a rise of temperature, above the previous level, on the third day.

TABLE IV.

TIME OF COMMENCEMENT AND DURATION OF REMISSION.

		Duration of Fever.					Total Cases.
		4 Days.	5 Days.	6 Days.	7 Days.	8 Days.	
		NUMBER OF CASES.					
Commencement of remission	1st evening	1	4	1	0	0	6
	2nd morning	19	12	5	0	0	36
	2nd evening	10	3	1	0	0	14
	3rd morning	13	19	1	2	1	36
	Total cases						92
		NUMBER OF CASES.					
Reported sick late in the attack		15	23	5	0	0	43
No obvious remission		22	18	2	1	0	43
		NUMBER OF CASES.					
Duration of remission	$\frac{1}{2}$ day	12	6	2	0	1	21
	1 „	15	7	2	1	0	25
	$1\frac{1}{2}$ days	12	15	4	1	0	32
	2 „	3	4	0	0	0	7
	$2\frac{1}{2}$ „	1	6	0	0	0	7
	Total cases						92

Cases reporting sick on the second evening, or later, naturally do not show the remission, and it is to be presumed that they report sick with the onset of the second phase. In such cases the existence of the first phase is only recognized by questioning, and it appears to be a feature of the disease that the first phase may be very mild as compared with the second phase.

During the course of the fever the evening temperature is usually about 1° higher than in the morning but with the exception of the remission between the two phases of the fever, normal is seldom reached except perhaps on the day before the final subsidence. The fever usually ends by lysis, but not infrequently there is an exacerbation on the last evening, in which case the fall occupies a few hours only.

In cases in which the second phase only lasts for a few hours, there is sometimes a rise of temperature to 99° F. on the fifth day. This rise is often so transient that it is only detected by a 4 hourly, or even by a 2 hourly, reading. In such cases, symptoms (*e.g.*, sore throat, headache or malaise), although alleviated with a return to normal temperature, persist until after the final rise, when they rapidly disappear.

Pulse.—The pulse rate from the onset of the disease is usually slowed by from 15 to 20 beats per minute. On cessation of the fever, the return to the normal rate is generally rapid, though in a number of cases it may remain relatively slow for as long as 1 week.

Central Nervous System.—The headache is characteristic, and is one of the most constant and typical symptoms. It appears at the commencement of the disease. It may be severe and is almost invariably confined to the frontal region, only very rarely does it extend to the temples and eyes or to the rest of the head. There is no photophobia.

There is no tendency to depression or delirium.

Pharynx.—From the onset the pharynx is acutely congested. Often this is painful, though many patients make no complaint and the throat condition is only discovered by examination. The congestion often spreads to the soft palate and posterior nares and remains till the end of the fever, when it rapidly clears up. On the soft palate there is a distinct and characteristic raised line of demarcation. There is no ulceration or membrane, and little if any mucus. In some cases only a mild hyperaemia is present, all intermediate stages being found. The intensity of the pharyngeal congestion bears little relation to the duration of the attack, except that cases of relapse more often have a milder congestion. The tonsils are never enlarged, but the mucous membrane covering them may share in the local congestion. Bacteriological examination of the throat clearly distinguishes the condition from a septic pharyngitis. There is no tendency for the ear to become involved.

Respiratory Tract.—Tracheitis, causing a hard dry cough without sputum, is frequently present. This appears to be an extension of the pharyngeal

condition and would account for the pain in the chest which one of the initial symptoms.

Loss of voice due to affection of the larynx has been observe

Digestive Tract.—Rarely, there is an early complaint of which would appear to be referred from the chest; vomiting n time of onset. Beyond this there is no involvement. Th enlarged.

Spleen.—In a proportion of cases the spleen becomes palpal enlarged to more than two fingers breadth below the costal mar to normal with convalescence.

Skin.—A transient erythematous rash appears in 2 per ce the first day, upon the back and, less often, upon the chest. Th more than a few hours. No later rashes are ever observed.

Lymphatic System.—Occasionally the glands draining th palpable. In no cases does suppuration occur. Other lymphati involved.

Genito-urinary System.—Febrile albuminuria may occur. has failed to detect organisms.

Eyes.—Suffusion never occurs. There is no photophobia o

Blood.—In the large majority of cases, a leucopenia, which r 3,000 white cells per c.mm., is established early. During the fever there is usually a slight increase in the percentage of larg which may even reach as high as 10 per cent., while the eosinop diminished and, in many cases, absent. Lymphocytes are n increased to 40 per cent. with a corresponding diminution of t In a few cases the while cells remain unaltered. With the fall of there is a slight eosinophilia with a rapid return of the total counts to normal. There is no change in the red cells or haemog

No organisms can be recovered from the blood. Special s made for those of the enteric group and for *Treponema recurrentis*

There is no alteration in agglutination reactions to the *Brucella melitensis*, *B. abortus*, *Bacillus proteus* X.

Malarial parasites cannot be found.

RELAPSES.

Of 411 cases, combatants and followers *admitted* in the year in Sialkot, fifty-three relapsed, a percentage of 12·90.

The relapse occurs most commonly on the sixth day, but r as the fifth morning or may be delayed till the tenth day. In a f may be a longer interval. Table V shows the day of onset of t

There is sometimes more than one relapse, and cases have

TABLE V.

INTERVAL BETWEEN ONSET OF FIRST RELAPSE AND ONSET OF INITIAL ATTACK.

Relapse on	5th	morning	after	initial	attack	...	3 cases
,,	5th	evening	,,	,,	,,	...	16 ,,
,,	6th	day	,,	,,	,,	...	27 ,,
,,	7th	,,	,,	,,	,,	...	5 ,,
,,	8th	,,	,,	,,	,,	...	0 ,,
,,	9th	,,	,,	,,	,,	...	1 ,,
,,	10th	,,	,,	,,	,,	...	1 ,,
				Total	...	...	... 53 cases

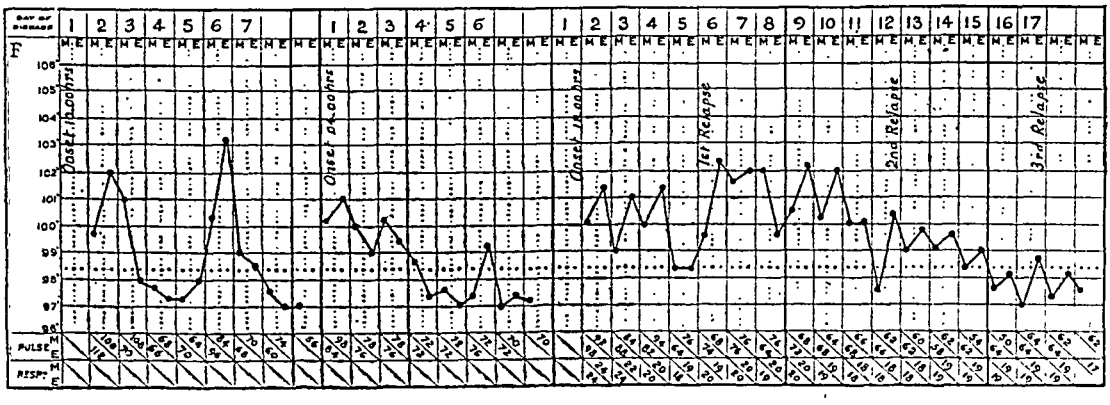
lasting in all for more than 1 month. These are believed to be caused by this disease, though examples were not met with in the series under analysis.

Of the fifty-three cases of relapse, forty-six relapsed once, four twice, two three times and one four times.

Table VI (p. 290) shows the duration of the initial attacks and also of the subsequent relapses of these fifty-three consecutive cases.

In reading charts of relapses it is necessary to bear in mind the effect of the remission as well as the interval between attacks. If this is not done, the true significance of the case will often be missed.

XVI. Recruit G. S. XVII. Naik P. S. XVIII. Syce L.



RELAPSE CASES.

TABLE VI.

DURATION OF INITIAL ATTACKS AND OF RELAPSES.

Duration.	Initial Attack.	Relapses.			
		First.	Second.	Third.	Fourth.
	Number of Cases.	Number of Cases.			
1 day	1	17	—	1	1
2 days	4	12	3	1	—
3 „	12	7	—	—	—
4 „	14	5	2	—	—
5 „	15	6	2	1	—
6 „	—	5	—	—	—
7 „	1	1	—	—	—
Unreliable history	6	—	—	—	—
Totals ...	53	53	7	3	1

(*d*) COMPLICATIONS.

During the winter months the respiratory tract is occasionally affected by a mild bronchitis. This complication, if found, tends to mask the remission and to cause a rise in the total leucocyte count by an increase in the neutrophils. Severe bronchitis has not been observed. The duration of the attack is not prolonged, and the bronchitis clears up immediately it is over. Dry ronchi are often heard in the chest. These are not to be taken as a sign of bronchitis; they appear to be caused by a spread of the tracheitis to the smaller bronchi. On rare occasions, a patch of consolidation, confirmed by X-ray examination, the size of the bell of a stethoscope has been found. This affects the leucocyte count in the same manner as bronchitis, does not effect the duration of the disease, appears to be of no prognostic significance and does not delay the patient's discharge from hospital.

In one case Pfeiffer's bacillus was isolated.

Very rarely true lobar pneumonia has supervened. This was the cause of the only fatality noted. This complication is so uncommon as to suggest accidental occurrence. In one case an old tuberculous focus in the lung was lighted up and occasionally malarial relapses may be determined.

No other complications have been observed.

(*e*) DOUBTFUL CASES.

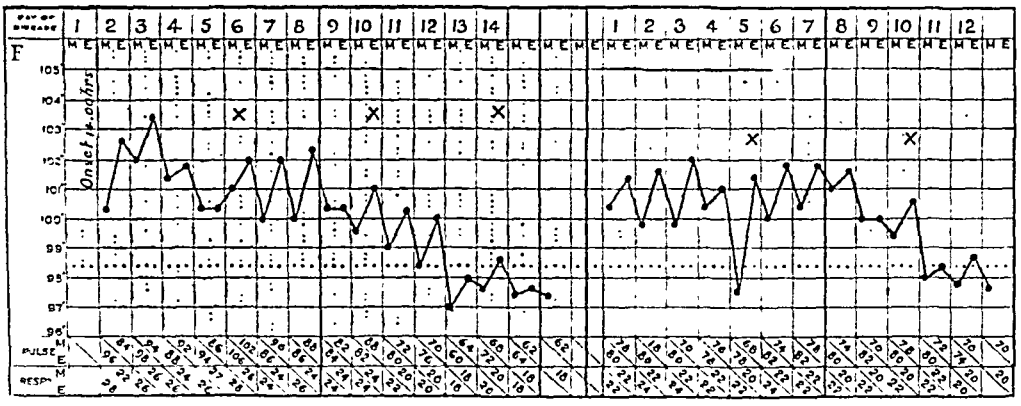
Certain doubtful cases have been excluded from the foregoing account. The general appearance is shown in Chart XIX. They have all the clinical

XIX.

Syce M. B.

XX.

Bugler S. A.



DOUBTFUL CASES. × = possible relapse.

features of the relapse cases described, but the temperature fails to fall between the attacks. It is well recognized that there may be no apyrexial period before a relapse in enteric fever. It would appear that in this disease, also, the apyrexial period may be missing. In the chart shown, peaks of pyrexia (marked ×) on the sixth and tenth days suggest this possibility.

Chart XX shows a similar case in which the temperature fell for a few hours. In this chart the slight rise (marked ×) on the fifth day of the relapse is also to be noted as suggestive of the commencement of a second relapse. It seems possible that in other cases the fall occurs at night and is unnoticed.

Some cases lasting 6 days show a rise on the last day. Possibly this is really the commencement of a relapse which fails to be maintained. If this is the case, it is evidence that a fall between the initial attack and the relapse is not necessary, and bears out the suggestion that these doubtful cases are, in fact, cases of relapse.

The patients never appear seriously ill and invariably make a rapid and uneventful recovery. All the bacteriological and serological examinations usual in cases of prolonged pyrexia are negative.

These doubtful cases occur amongst undoubted cases. They include a large number of the cases of pyrexia of uncertain origin met with in the north of India.

5. Diagnosis, Prognosis and Treatment.

Diagnosis.

The diagnosis presents no difficulty if the salient features already described are borne in mind. It is necessary to recognize the fact that the throat condition is not the cause of the disease but is a symptom only. Correct diagnosis is very

desirable to avoid anxiety in cases in which the fever is prolonged by relapses, to spare patients the unnecessary discomfort of repeated blood cultures, etc., and to escape avoidable interference with men's duties caused by treatment for malaria. Recognition of this disease is also necessary in order that its causation and prevention may be worked out.

The usual diagnoses made are: pharyngitis, sandfly fever, influenza, bronchitis, dengue, clinical malaria, common cold or tonsillitis.

Fevers of the enteric group, malaria, relapsing fever and typhus are excluded not only by laboratory methods, but also by the clinical course and symptoms. The discharge of a septic throat is absent. Common cold is excluded by the absence of nasal discharge; any blocking of the nasal passages is due only to swelling of the mucous membrane of the posterior nares which has spread up from the pharynx. Tonsillitis also is excluded by the absence of follicular patches; the swelling, if present, is of the mucous membrane only, though this may of course be covering a chronically enlarged tonsil. If bronchitis is present, a study of the case will show that this is a complication, not the primary disease.

The main differential diagnosis lies, therefore, between the disease under discussion and dengue, sandfly fever and influenza. This disease is common in areas in which dengue rarely or never occurs. It occurs in the absence of *Aëdes argenteus*. The rash of dengue is never seen, nor is the desquamation. Severe pains of joints and muscles are never experienced. There is no injection of the conjunctivae. The fulminating course of an outbreak of dengue is never encountered. The remission between the two phases of the fever in this disease tends to be at least 24 hours earlier than in dengue. This disease lasts longer than sandfly fever and the whole course presents an entirely different clinical picture. It is also common at seasons when *Phlebotomus papatasii* cannot be found and in places in which sandfly fever is very rare. Influenza is excluded by the duration of the fever, the invariable mildness of the effects, the rapid convalescence, and the slow pulse. The type of influenza is apt to vary from year to year; the type of this disease does not. The absence of depression and the lesser degree of malaise also clearly distinguish this disease from the three preceding diseases of which depression is a distinctive feature.

Prognosis.

Uncomplicated cases invariably recover.

Treatment.

The treatment of the disease is symptomatic. No specific treatment is known. Atebrin, plasmoquin and sulphostab are without effect.

6. Etiology.

The etiology of the disease is still in doubt.

(*a*) The many resemblances to the sandfly-dengue group strongly suggest

an insect vector. Attempts to find such a vector have been made with only very doubtful success.

A winged vector is not possible. At Razmak cases occur in the coldest season with snow on the ground, in the absence of mosquitoes and sandflies. The lines of British units were sandwiched between those of affected Indian units, separated by less than 100 yards, yet no cases were observed in British troops. In the Punjab, the incidence is greatest in the colder months when fresh infections of malaria are rare. The mosquito and sandfly cannot, therefore, be vectors.

The louse is not the vector: thorough disinfestation of affected units has been carried out on five occasions without any influence upon the incidence of the disease. Attempts to transmit the disease by lice have failed. In six cases lice, in batches of ten, were fed upon patients, from the first to the fourth day of disease, and thereafter fed upon volunteers (who had no history of recent illness) until the death of the lice, which occurred in five cases on, or before, the sixth day, and in one case on the ninth day. In one case lice were fed upon a patient on the first day of disease and then released in the clothing of an already louse-infested volunteer. He wore the same clothes and remained infested for 3 weeks. None of these volunteers developed the disease.

It seems unlikely that the flea is the vector. The incidence of the disease does not show the seasonal and climatic variations which would be expected if this were the case.

It appears possible that the bed-bug is the vector; the disease is present throughout the year, a prevalence which is consistent with this. Attempts to transmit the disease by bed-bugs have been made. At Ambala, in forty cases, bed-bugs (*Cimex rotundatus*), obtained from houses free from history of illness were fed, in batches of ten, upon healthy men as controls. In no case did the controls develop fever. The bed-bugs were then fed upon patients on either the first, second or third day of the disease, and thereafter upon volunteers every third or fourth day for varying periods up to 1 month. These volunteers were men who were in hospital for minor injuries and, therefore, unlikely to be exposed to infection. In two cases volunteers developed fever after being bitten. In one case the fever lasted a few hours only without typical symptoms, but in the other a nearly typical attack of the disease with relapse followed. The bed-bugs had been infected 9 and 3 days previously, and the volunteers had been in hospital for 5 and 17 days. The one might have been infected before arrival at hospital and the other might conceivably have broken out of hospital and been infected elsewhere. Similar attempts were made in Sialkot using both *C. rotundatus* and *C. lectularius*, but without success.

The disease is not spread by food, milk or water, since men in one barrack room may suffer from the disease, while others of the same caste and company fed from the same cookhouse under precisely the same conditions, but living n another barrack room, may be entirely unaffected.

(*b*) Attempts have been made to effect direct transmission of the disease. In one case, 5 c.c. whole blood from a patient on the third day of disease was injected subcutaneously into a volunteer; the necessity for taking the obvious precautions prevented this being carried out earlier. In another case filtered serum from a patient on the second day was injected. In another case a direct throat to throat swabbing was tried. None of these attempts was successful.

(*c*) Throat washings in normal saline were taken, passed through a Seitz-Werke filter and inoculated into a rabbit. The temperature rose 2° F. and the rabbit went off its food the same night, but thereafter returned to normal.

(*d*) The incidence of the disease appears to bear no direct relation to climatic conditions, such as temperature, rainfall or relative and absolute humidity. In Razmak the incidence appears to be greater in the temperate hot weather than in the extreme cold, whereas in the plains of the Punjab the incidence is somewhat greater in the cold weather months, especially during December and January. It is endemic in all units throughout the year; units seldom go for more than 3 weeks at a time without a case reporting sick. At irregular intervals the incidence in a unit increases into a minor epidemic.

The course of incidence in a unit is that sporadic cases occur, scattered throughout the squadrons or companies, but these cases do not communicate the disease to other men; then, from no apparent cause, cases occur more frequently in a barrack or barrack room, with any interval from a few days to 3 weeks between cases. Occasionally two cases may go sick on the same day in the same barrack room. There is no constant interval between cases. The spread is slow, and some 6 to 10 weeks may elapse before the incidence subsides to the normal rate of sporadic cases. A barrack room may go for many months without a case, and for as much as a year without an epidemic. An instance has occurred in which, in a barrack, divided into three barrack rooms of twenty men each, one room had as many as eleven men attacked in the course of a few months while in the other two rooms there were no cases, in spite of the fact that one of them had men of the same caste as the men attacked, and although they all mixed on parade. Such epidemics in one unit do not correspond with epidemics in other units, nor usually do those in the different squadrons or companies of the same unit.

It is interesting to note that though no precautions are taken with cases in hospital, the disease never spreads to other patients nor are those in attendance on the sick, medical officers and nursing orderlies, more prone to the disease than others.

Troops in camp appear less likely to contract the disease than those in barracks. In one case, amongst 450 reservists in camp for 5 weeks in Sialkot in February, ten to a tent, only six cases occurred, although the disease was prevalent in the parent unit in barracks only a few hundred yards distant. These cases were all in adjacent tents, but no two cases occurred in any one tent.

British officers of Indian units do not often contract the disease; when

married officers do so, it does not spread to their families. In the only case hitherto seen of an officer's wife having contracted the disease, she developed it within 36 hours of the officer and so both presumably contracted the infection from the same source, though this could not be traced. It is also unusual for Indian officers to be infected.

7. Immunity.

Second attacks are not uncommon. One attack does not give immunity to further attacks. Second attacks are rare within 9 months, but have been noted with an interval of 6 weeks only. It appears not improbable that the varied types of the disease are due to a partial immunity due to previous attacks. The general health of the individual appears to have a direct bearing upon the susceptibility to attack. It is especially noticeable that those with a haemoglobin of less than 70 per cent. appear to be more susceptible than those above that figure, and tend to have more severe attacks.

8. Discussion.

The most striking features of this disease are the saddle-back nature of the fever, frontal headache, sudden onset, sore throat and rapid convalescence. The complete absence of pain in the eyes and joints, of coryza, of late rash and of suffusion of the conjunctivae are also to be noted, as are the mildness and lack of persistence of the muscle pains. The remission in the earlier part of the course is typical, but, should this be absent, the diagnosis still holds.

That all the cases showing different durations of fever are caused by the same disease is clear from the following points :—

1. The tendency of cases of short duration to relapse about the sixth day. An initial attack of 1 or 2 days may give rise to a relapse of 5 days.

2. Not only do cases of various duration present the same clinical appearance, but as regards duration they merge into one another imperceptibly.

3. The cases of various degrees of severity and duration all appear in due proportion during the same epidemics and from the same companies and barrack rooms, as they do also in different epidemics and at different times of the year.

These points can only be fully brought out by a close study of the incidence and by a careful history of the precise time as well as date of onset. It seems probable that the varying duration is caused by the suppression of the second phase of the fever. The tendency of the second phase to suppression is well recognized in dengue, while in sandfly fever it is almost universal ; actually, the point must be raised whether the cases of sandfly fever in which a second phase has been noted were not, in fact, cases of the disease now described.

A proportion of cases report sick late in the disease. It appears that these are either cases in which the first phase is mild, or in which the first phase is short and the remission between the phases is prolonged. Such cases report

sick with the onset of the second phase, and, if the patient is not questioned carefully, the existence of the first phase is overlooked.

The disease is invariably true to type, within the limits described, in whatever locality or in whichever year or season of the year it is encountered. The distribution is widespread and it certainly exists in the country villages since men on leave in them from other stations have reported for treatment. Moreover men travelling from stations other than those in which the disease has been studied have been attacked during the journey

The method by which the disease is spread still remains in doubt. No connecting link between cases can be discovered, except that minor epidemics appear to arise in individual barracks. No reasonable explanation can be given as to why sporadic cases should occur at one time in a barrack room without the disease spreading, while at another time a minor epidemic breaks out. Nor is it clear why such minor epidemics should appear, irrespective of climatic or other conditions, in one squadron or company, and not in another when the disease in endemic in all. Experience at Razmak and the increased incidence of the disease in the cold weather in the plains, make a winged vector impossible, but, from analogy with the dengue-sandfly group, an insect vector still seems probable. This suggestion is strengthened by the very irregular interval between cases. The bed-bug has on several occasions been a suspected carrier of disease ; in this case also it seems the most likely. Perhaps there is some unknown factor which interferes with the success of attempts to use it as a vector. The attempts made to transmit the disease by this method are, however, against rather than in favour of the bed-bug as a vector. Ticks are not found in barracks.

Droplet infection is possible. The fact that the disease does not spread in hospitals can be explained by the fact that hospital cases are confined to well-spaced beds during the time that they may be regarded as infective. This method of infection, however, seems improbable : were it responsible, a more regular interval between cases would be expected.

9. Conclusion.

A very large number of cases of mild fever characterized by a saddle-back temperature, frontal headache, slow pulse and sore throat have been observed in various stations in northern India.

The clinical picture suggests a resemblance to the sandfly-dengue group, but there are many definite differences.

The disease has a distinct tendency to relapse and it is these relapses which form a large proportion of the otherwise undiagnosed fevers common amongst Indians in the north of India.

The existence of an insect vector, perhaps the bed-bug, is possible.

There can be no doubt that this is a disease *sui generis* hitherto undescribed, and to it the name " sellar fever " is now given, in allusion to the typical course of the fever.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

NOTE ON ACUTE HEPATITIS AND YELLOW FEVER IMMUNIZATION.

BY

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AND

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From time to time reactions have been described following immunization with attenuated viruses such as vaccinia and fixed rabies and, as is well known, various phenomena, grouped under the term " serum sickness," may follow the injection of therapeutic sera. Most of these reactions follow the injections at an interval of at most 7 to 12 days. If the occurrences described in the present note are other than fortuitous it would appear that sequelae to immunization may be delayed for as long as 2 to 7 months after immunization. The sequelae referred to are attacks of acute hepatitis, with symptoms resembling those of common infective hepatic jaundice, coming on at intervals of 2 to 7 months after yellow fever immunization. The term " common infective hepatic jaundice " is here used as a synonym for " epidemic catarrhal jaundice," a disease associated with a mild hepatitis and distinguishable as HURST and SIMPSON (1934) point out, from the rarer condition of true catarrhal jaundice. During the past $4\frac{1}{2}$ years approximately 2,200 persons have been immunized against yellow fever. Careful records have been kept of both the immediate results of the inoculations and of any remote illnesses from which the persons immunized may have suffered. In all, fifty-two cases of jaundice have been traced. Two of the patients who became jaundiced were successfully operated on for gall stones and gave a history pointing to involvement of the gall bladder which antedated the yellow fever immunization. In two other instances the patients shared a meal of curried prawns. Symptoms of acute gastro-intestinal derangement followed a few hours later and both patients subsequently developed a slight jaundice. The symptoms, suggestive of some form of food-poisoning, resembled those of the jaundice met with during the war in Gallipoli.

There is no reason to think that the occurrence of jaundice in these four persons was in any way associated with the yellow fever immunization.

In the forty-eight other patients the symptoms exhibited a striking uniformity. After a few days of slight malaise, with loss of appetite, nausea and occasional vomiting, the skin and conjunctivae were noticed to be jaundiced and the urine dark coloured. Sometimes the stools were pale in colour. The symptoms then began to clear up, though the jaundice, weakness and mental depression disappeared more gradually. No case has so far been fatal and in no case was there any complication. Of the forty-eight patients, six only were women, although of the 2,200 persons immunized nearly 400 have been women The persons affected varied in age from 22 to 58 years Six cases occurred in this country, fourteen in Egypt and the Anglo-Egyptian Sudan, the remainder in the British West African colonies, the Gambia, Sierra Leone, Nigeria and the Gold Coast.

The interval between the yellow fever immunization and the onset of the jaundice was almost exactly 2 months in 26, between 2 and 3 months in 12, between 3 and 4 months in 8, and over 4 months and just under 7 months in 2 cases.

Clinical Histories of Typical Cases.

Case 1, inoculated on 3.12.36 with tissue culture pantropic yellow fever virus and 28 c.c. human yellow fever immune serum : no reaction occurred after the inoculation and 3 weeks later his blood when tested for immunity to yellow fever protected in a dilution of 1 in 16. He left for Northern Nigeria at the end of January, 1937. On 18.2.37, he felt a little out of sorts but at no time had any fever. Two days later it was noticed that he was jaundiced. The jaundice deepened for a few days and was accompanied by anorexia and lack of energy. Then it gradually disappeared together with the other symptoms and at the end of 4 weeks he had completely recovered.

Case 7.—Physician, aged 30 years, was immunized against yellow fever on 21.4.36 with tissue culture pantropic yellow fever virus and 30 c.c. of human yellow fever immune serum and 5 c.c. of concentrated monkey yellow fever immune serum. Apart from a slight serum rash at the point of injection of the monkey serum, there was no reaction to the injection. The yellow fever immune body titre of the serum a month later was found to be 1 in 16 when tested by the mouse protection test. On 27.5.36 the patient was given a second inoculation of pantropic yellow fever virus alone. No reaction followed and a fortnight later the immune body titre by the mouse protection test was 1 in 64.

For some 6 weeks before the onset of illness the patient had been staying at a farm in a rural part of Sussex. About 16.7.36 a feeling of uneasiness developed as though he was carrying a weight in the epigastrium. This continued for 4 days when he felt a little faint. On this day he was given 0·5 c.c. T.A.B. vaccine. The following day he had nausea on rising and pains in the limbs but no temperature. He went to bed after a light lunch. On 23.7.36 the general lassitude was more marked and the urine was very dark coloured. The temperature in the evening was 99·6° F. It did not rise again above 99° F. throughout the course of the illness. The patient vomited in the evening. On 24.7.36 he felt better but for the first time noticed that his conjunctivae were jaundiced. Thereafter he slowly improved. He was admitted to the Hospital for Tropical Diseases on 28.7.36 under the care of Dr. N. H. Fairley. An examination of the heart and lungs revealed nothing abnormal and there was no tenderness over the abdomen. Examination of the blood showed haemoglobin 100 per cent. : leucocytes 6,600 per c.mm. The differential count showed polymorphonuclear leucocytes 47 per cent., small lymphocytes 45 per cent.,

mononuclears 6 per cent., eosinophils 1 per cent. and basophils 1 per cent. The stools were pale in colour and showed an absence of ova and protozoa. Urine, sp.g. 1,018, reaction acid, albumin a trace, sugar nil, red blood corpuscles absent; leucocytes and epithelial cells a few: casts nil. Bile salts, bile pigments and urobilin positive, van den Bergh: direct reaction positive (biphasic): indirect reaction 19 units of bilirubin. Blood urea = 25 mgm./100 c.c. The patient made an uneventful recovery. An examination of the blood serum for immune bodies to leptospira carried out by Major H. C. BROWN, was negative. Attempts to demonstrate the presence of yellow fever virus in the serum by the subcutaneous inoculation of rhesus monkeys and the intracerebral injection of mice were negative. The yellow fever immune body titre of the serum examined during the illness and a fortnight after convalescence was complete was constantly 1 in 64.

Case 9.—Laboratory assistant aged 22 years: was immunized with the neurotropic strain of yellow fever virus and 20 c.c. of human yellow fever immune serum on 29.11.33. A week later he complained of a severe headache, slight photophobia and hypersensitivity of the skin. The temperature rose to but did not exceed 100·4° F. He felt out of sorts for 5 days. He remained in good health up to 3.3.34 when he had a bad cold in the chest with cough: he occasionally felt slight nausea but did not actually vomit till 10.3.34, when he noticed that his urine was dark in colour. His appetite was poor and he suffered off and on from flatulence. On 12.3.34 he noticed that he was jaundiced. On examination no abnormality was noticed except tenderness in the right hypochondrium. The stools were well formed, of normal colour and neutral reaction: the fatty acid values were low. The van den Bergh test gave direct a delayed positive, indirect 10·5 units of bilirubin: the urine sp.g. 1,024, acid reaction—albumin slight trace, occasional leucocytes and epithelial cells: bile salts, bile pigments and urobilin were all present. The patient made an uneventful recovery and on 4.4.34 the urine give negative reactions for bile pigments and salts with but a slight trace of urobilin.

The yellow fever immune body titre of the serum tested 5 weeks after the yellow fever inoculation by the mouse protection test was 1 in 64: tested a year later it had fallen to 1 in 16.

For clinical details of the above cases I am greatly indebted to Dr. N. HAMILTON FAIRLEY, Physician to the Hospital for Tropical Diseases.

Case 18.—Physician, was inoculated with pantropic tissue culture virus and 32 c.c. of human yellow fever immune serum on 31.10.36. Beyond a slight feeling of tiredness he experienced no reaction to the inoculation and his serum 4 weeks after inoculation was found to protect in a dilution of 1 in 32. During the second week in January he felt out of sorts, was easily tired and had a poor appetitte: on 16.1.37 he noticed that he was jaundiced. With the onset of jaundice the subjective symptoms improved but the anorexia and evidence of jaundice did not completely disappear for nearly 6 weeks. The faeces were pale and clay coloured, the urine very dark in colour with bile salts, bile pigments and urobilin. A differential blood count showed polymorphonuclear leucocytes 53, small lymphocytes 42, mononuclears 4, eosinophils 1. The serum tested by Major H. C. BROWN, for immunity to leptospira gave negative results. The yellow fever immune body titre remained constant throughout and no yellow fever virus could be demonstrated by inoculation of either whole or diluted blood serum into monkeys and mice. Blood cultures were bacteriologically sterile.

Case 21.—Company director, was immunized against yellow fever on 10.11.36 with pantropic tissue culture yellow fever virus and 40 c.c. of human yellow fever immune serum. His serum protected against yellow fever virus in a dilution of 1 in 32 3 weeks after inoculation. He sailed for West Africa on December 16th, 1936, and after visiting various parts of West Africa arrived at Garua, French Cameroons in early January: after spending a week or 10 days he moved on to Northern Nigeria and towards the end of January began to feel out of sorts with loss of appetite. There was no fever. This lasted for 4 days when he noticed that his stools were light in colour and his urine dark. Later he was told that his skin and conjunctivae were jaundiced. He did not have to go to bed but continued to feel below par for the next 3 months. His serum tested in May, 1937, protected mice in a dilution of 1 in 64. Major H. C. BROWN was unable to demonstrate leptospiral immune bodies in the serum.

Methods of Preparing Yellow Fever Virus and Immune Serum.

Before discussing the cause of these cases of hepatitis and their relationship if any, to the preceding yellow fever immunization, it is necessary to recapitulate the various modifications (*cf.* Findlay, 1936) that have been made in the method of yellow fever immunization. The method, first introduced by Sawyer, Lloyd and Kitchen (1932), consisted in the injection of human immune yellow fever serum in doses of 0·5 c.c. per kg. of body weight, followed some 2 to 3 hours later by an injection of 0·5 c.c. of a filtered 20 per cent. suspension in normal human serum of mouse brain infected with the neurotropic strain of yellow fever virus. The immune serum was obtained either from persons who had suffered from clinical attacks of yellow fever or from persons such as laboratory assistants who had been artificially immunized against yellow fever. All donors were Wassermann negative. The serum was preserved by the addition of 0·2 c.c. of tricresol mixed with 0·2 c.c. of ether and stored in sealed glass ampoules, so that no loss of tricresol could occur on standing: before bottling it was filtered through a Seitz K filter and was tested for bacterial sterility by incubation in glucose broth under aerobic and anaerobic conditions. Later, owing to the difficulties of obtaining sufficient supplies of human serum, an attempt was made to use serum from hyperimmunized horses (Pettit and Stéfanopoulo, 1933) and also hyperimmunized monkeys. The same preservatives and the same precautions as to bacterial sterility were used as in the case of human sera.

Cases of jaundice have been seen in association with the use of human immune serum prepared both in these laboratories and in the laboratories of the International Health Division of the Rockefeller Foundation in Brazil (ten cases), after the use of hyperimmune horse serum prepared in the Pasteur Institute, Paris (thirteen cases) and after the use of hyperimmune monkey serum (two cases) prepared in these laboratories. The smallest dose of serum which has been followed by jaundice is 10 c.c.

The virus component used from 1932 to the middle of 1936 consisted of a 20 per cent. suspension in normal human serum of mouse brain infected with the neurotropic strain of yellow fever virus. The normal human serum employed was filtered through Seitz K filters and tested for sterility before use. The mice used in the production of this vaccine were of a specially selected strain which was periodically tested for freedom from intercurrent infection. The vaccine before use was filtered through a Seitz K filter and tested both aerobically and anaerobically for bacterial contamination. Use has been made of neurotropic yellow fever vaccine prepared in these laboratories and in the yellow fever laboratories of the International Health Division of the Rockefeller Foundation in New York. Jaundice has occurred after the use of vaccines prepared in both laboratories. From the middle of 1936 onwards an attentuated pantropic strain of virus has been exclusively used. This attenuated strain of ordinary yellow fever virus, first prepared by Lloyd (1936), was to begin with grown in tissue culture flasks

containing minced mouse embryo tissues in normal serum Tyrode solution. Later (FINDLAY, 1936), minced chick embryo was substituted for the mouse embryo. Jaundice has occurred after the use of vaccines prepared from virus grown in either mouse or chick embryo and prepared both in New York and London.

POSSIBLE EXPLANATIONS OF THE OCCURRENCE OF HEPATITIS FOLLOWING YELLOW FEVER IMMUNIZATION.

An explanation of the occurrence of hepatitis as a sequel to yellow fever immunization is by no means easy. The following possibilities suggest themselves :—

(1) *Coincidence.*—Since epidemics, and apparently sporadic cases, of common infective hepatic jaundice are by no means rare in the general population it was at first thought that the appearance of an occasional case of jaundice following yellow fever immunization was purely fortuitous. Nevertheless the fact that between 2 and 3 per cent. of those inoculated over a period of 4 years have developed jaundice suggests that a positive correlation exists between the inoculation and the jaundice.

The following facts must also be taken into consideration, (*a*) the invariable interval of at least 2 months between the inoculation and the onset of jaundice, (*b*) the occurrence within a few days of each other of jaundice in three out of five immunized persons in a mining camp in Sierra Leone : no non-immunized persons developed jaundice, (*c*) the occurrence within a few days of each other of jaundice in seven out of thirty-two immunized persons in a camp in the Anglo-Egyptian Sudan almost exactly 2 months after immunization : no case of jaundice occurred at the same time in 198 non-immunized persons in the same camp. In another camp of twenty persons immunized one became jaundiced, in 798 not inoculated no case of jaundice occurred.

On the other hand in order to obtain a rough idea of the frequency of jaundice in the general population the last one hundred people coming up for inoculation have been asked whether they have suffered from jaundice during the past 10 years. Four persons reported that they had had jaundice during this period, two of them in Africa.

(2) *Jaundice may be caused by the yellow fever virus.*—The next suggestion was that the jaundice was directly due to the yellow fever virus and that the illness was a modified form of yellow fever, the virus having gained entrance into the liver where it remained unneutralized till the immune serum had been excreted, when it again became active. The following facts contradict this suggestion : (*a*) the clinical symptoms ; these closely resemble those associated with common infective hepatic jaundice and differ from those of yellow fever in the almost complete absence of fever, the absence of headache and backache, the frequent paleness of the stools, the long continuance of the jaundice, (*b*) failure

to obtain virus from the blood, (*c*) lack of increase in the yellow fever immune body titre of the serum as a result of the attack of jaundice. All persons who developed jaundice with one exception had been shown *before* the onset of the hepatitis to have yellow fever immune bodies present in their blood, (*d*) failure of all efforts to obtain yellow fever virus from the tissues of animals after the development of yellow fever immune bodies (HINDLE, 1932), (*e*) development of jaundice in a woman following the injection of yellow fever immune serum and neurotropic yellow fever virus, the patient having already suffered from an attack of yellow fever some 8 weeks before the date of her immunization. Her serum taken immediately before injection of the serum-virus mixture was shown by the mouse protection test to protect in a dilution of 1 in 128. Two clinical attacks of yellow fever in the same individual have never been recorded nor is there any record of jaundice or hepatitis coming on 2 months after recovery from naturally occurring yellow fever.* (*d*) After the injection of yellow fever immune serum into animals BAUER (1930) has shown that immune bodies have disappeared 14 days later. If yellow fever virus were lying latent in the liver it is curious that no cases of jaundice occurred till 60 or more days after inoculation.

(3) *The jaundice may be due to some organism injected with the virus or serum.*— It is conceivable that some extraneous organism might have gained entrance when the yellow fever immune serum or the virus was injected. Since both the yellow fever immune serum and the virus suspension were filtered through Seitz filters and were bacteriologically sterile after filtration the hypothetical organism would almost certainly have to be a virus. If the virus were introduced with the serum it must be present in the serum of apparently healthy human beings both in England and South America and in the serum of horses and monkeys and it must not be killed by the addition of 0·2 per cent. tricresol and 0·2 per cent. ether. If the hypothetical virus were injected with the yellow fever virus then the virus must have been originally present in the brains of mice in New York and London or in the monkeys tissues from which the pantropic yellow fever virus was originally obtained. No evidence from other sources has been obtained that mice or rhesus monkeys harbour a virus capable of producing jaundice in human beings. If a hypothetical virus pathogenic for man were directly injected with the virus or serum inoculum it is surprising that under 3 per cent. of persons developed symptoms. Although the presence of a hypothetical virus cannot be entirely excluded the evidence against it is very great. All serum used in growing the virus or in making the vaccine is now being inactivated before use.

*Since the above was written we have seen a European, A. M., who suffered from a typical attack of yellow fever in November, 1933, on the Gold Coast. Two other Europeans were attacked at the same time, one of whom died. Between two and a half and three months after his attack of yellow fever A. M., while still in West Africa, suffered from an attack of jaundice with clay coloured stools and symptoms in every way similar to those reported above.

(4) *The jaundice may be due to some toxic substance contained either in the virus or serum inoculated.*—The possibilities which suggest themselves are :—

(*a*) Foreign proteins derived either from the mouse or chick tissues included in the virus inoculum.

(*b*) Foreign proteins contained in the immune serum.

(*c*) Tricresol.

(*d*) A toxic compound of tricresol and protein.

In regard to the toxic possibilities of the very small dose of mouse or chick protein injected with the virus component it seems improbable that the small amounts of foreign protein would in themselves cause an hepatic lesion. Cresol in large doses is known to have a toxic action on the liver, an action which it shares with other phenolic compounds, but the quantity (0·2 per cent.) actually injected into each individual has never exceeded 0·1 c.c. Many tens, if not hundreds, of thousands of persons must have been injected with serum containing tricresol without developing jaundice. Nevertheless experiments are now in progress to determine whether on standing tricresol forms with proteins compounds which might possibly injure the liver.

(5) *The jaundice may be due to some infection acting on a damaged liver.*—The symptoms recorded in the cases of jaundice following yellow fever inoculation are, as already mentioned, very similar to those of common infective hepatic jaundice, the disease formerly, though incorrectly, termed epidemic catarrhal jaundice. Common infective hepatic jaundice can be distinguished from leptospiral jaundice by the failure to infect guineapigs from the blood or urine, by the absence of agglutinins to leptospira in the serum, and by the differential leucocyte count which in infective hepatic jaundice shows an increase in lymphocytes, while in leptospiral jaundice there is commonly an increase in polymorphonuclear leucocytes.

Owing to the similarity in the symptoms of common infective hepatic jaundice and the hepatitis following yellow fever immunization the question naturally arose as to whether any of the persons who contracted jaundice following yellow fever injections could possibly have been infected with the agent, almost certainly a virus, of common infective hepatic jaundice. Common infective hepatic jaundice is by no means a rare disease in this country (*cf.* MORGAN and BROWN, 1927; FINDLAY, DUNLOP and BROWN, 1932; BASHFORD, 1934; FRAZER, 1935; BARBER, 1937; LISNEY, 1937) and in America (BLUMER, 1923). It has also been frequently noted in Egypt, the Anglo-Egyptian Sudan (*cf.* PRIDIE, 1936), where incidentally Weil's disease has never been recorded and West Africa (KUMM, 1932; FINDLAY, DUNLOP and BROWN, 1932). A widespread epidemic has recently been reported in Finland by WICKSTROM (1936). The chief pathological change is an acute but mild hepatitis (EPPINGER, 1922; FINDLAY and DUNLOP, 1932; GASKELL, 1933; HURST and SIMPSON, 1934). The incubation period has been shown by PICKLES (1930, 1937), and others to be about 4 weeks and the infectious stage would appear to be the preicteric period

when the disease is probably spread as a droplet infection. It is thus by no means easy to determine a clear association between sporadic cases of hepatitis and epidemics of common infective hepatic jaundice. Nevertheless in two instances in this series it has been possible to trace a connection between an outbreak of infective hepatic jaundice and the onset of jaundice in an inoculated person.

Case 18.—This physician, 4 weeks before the onset of his illness, had examined a number of children suffering from common infective hepatic jaundice in the out-patient department of a children's hospital.

Case 21.—This patient on arriving at Garua, French Cameroons, found that a European employee of his firm had just gone to bed with jaundice. This employee, O.H., who has since returned to England, reports similar symptoms to those described in *Case* 21 ; he also informed us that during January, 1937, there was a widespread outbreak of infective hepatic jaundice in the town of Garua. O.H. had never been inoculated against yellow fever and his serum failed to show either yellow fever or leptospiral immune bodies. *Case* 21 developed jaundice after an incubation period which is consistent with his having contracted the infection at Garua.

A third instance of association between common infective hepatic jaundice and post-inoculation jaundice is reported to us by Dr. G. J. STÉFANOPOULO who in 1935 immunized 102 white persons against yellow fever at Pointe Noire, French Gabon (*cf.* STÉFANOPOULO, 1937). Yellow fever hyperimmune horse serum and neurotropic yellow fever virus were employed. Two persons developed jaundice between 2 and 3 months after the inoculation, but as cases of infective hepatic jaundice were present in the town in non-inoculated persons no particular significance was attached at the time to the occurrence.

Even if the cases of post-inoculation jaundice are really cases of common infective hepatic jaundice, and the occurrence of cases of post-inoculation jaundice in little groups in Bathurst, Gambia, in a mining camp in Sierra Leone and in a camp in the Anglo-Egyptian Sudan is suggestive of some common source of infection, it is necessary to explain why persons who have received therapeutic inoculations against yellow fever should be more prone than the uninoculated to infection with the organism of infective hepatic jaundice. It is suggested that a possible explanation lies in a previous weakening of the liver by some hepatotoxic substance contained in the inoculum in insufficient quantities to produce by itself any demonstrable action. In one case in this series the onset of jaundice appears to have been precipitated by an operation for hernia ; the anaesthetic was A.C.E. As is well-known, two toxins acting together in small doses are more likely to produce extensive liver necrosis than a single toxin. OPIE (1910) for instance showed that while the effect of chloroform on the liver was very variable, the combined injection of living bacteria and chloroform produced much more constant and severe damage. Similar results on the liver were obtained by HURST and HURST (1928) on the combined effects of manganese chloride and

Bacillus coli infections, and by FINDLAY, DUNLOP and BROWN (1932) on the combined effects of arsphenamine and bacterial infections.

In human beings numerous outbreaks of jaundice have occurred in persons undergoing arsphenamine treatment. These outbreaks have, it is suggested, been due to the causal agent of common infective hepatic jaundice acting on the liver damaged by arsphenamine; in some instances it has been found that contact cases not receiving arsphenamine have also suffered from hepatitis. Earlier literature is summarized by FINDLAY (1930). A more recent outbreak is described by RICHARDS (1933). From 1931 onwards jaundice appeared in patients receiving antisyphilitic treatment in a clinic until finally one in every three patients developed symptoms. In all over 120 cases were seen. Female patients were relatively immune. Eventually one patient developed jaundice who had not received arsenical treatment for over a year, while another patient with jaundice had received only bismuth. It was found that the patients were forced to wait in a badly-ventilated corridor. When excessive crowding was avoided and all patients were made to wait in a large well-ventilated Out-Patient Hall the incidence of jaundice immediately fell.

MURRAY (1930) reported that of patients treated with acriflavine for gonorrhoea no less than 11 per cent. developed symptoms indistinguishable from those of infective hepatic jaundice (epidemic catarrhal jaundice) 8 or more weeks after cessation of the treatment.

DISCUSSION.

The evidence which has been obtained may be briefly summarized. Cases with symptoms closely resembling common infective hepatic jaundice have occurred 2 to 6 months after yellow fever immunization in from 2 to 3 per cent. of 2,200 persons immunized against yellow fever. Jaundice has occurred in those persons who have received immune serum and virus and also in persons who have received virus alone (five cases). The jaundice does not appear to be due to a form of yellow fever since no rise in the yellow fever immune body titre of the serum occurred during or after the illness and also because symptoms of jaundice occurred in a woman who had suffered from a natural attack of yellow fever and had a very high serum immune body titre already present when she received injections of virus and serum.

The occurrence of jaundice after yellow fever immunization is analogous to the occurrence of outbreaks of jaundice following antisyphilitic treatment or injections of acriflavine.

In two of the forty-eight cases discussed the attack of jaundice came on 4 weeks after exposure to infection from the organism of common infective hepatic jaundice. The incubation period of common infective hepatic jaundice is 4 weeks.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

THE LEUCOCYTE PICTURE IN IRAQ.

BY

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INTRODUCTION.

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PERCENTAGE STANDARDS EXTRACTED FROM FORTY-THREE TEXT-BOOKS.

	Polymorphs.	Eosinophils.	Basophils.	Lymphocytes.	Monocytes.
Extreme ranges	50–75	0–6	0–1	15–45	0·2–15
Average maximum	71·1	4·5	0·6	30	3·8
Average minimum	64·5	1·5	0·4	20·8	6·8
Mean average	68	2·6	0·5	24·2	4·8

*The paper is published by permission of the Air Ministry and the Director-General of Health, Baghdad.

Thanks are due to Dr. A. C. AITKEN for advice on some of the statistical points.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

THE LEUCOCYTE PICTURE IN IRAQ.

BY

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INTRODUCTION.

Differential counts are so commonly used in clinical practice that it is important to have accurate standards for comparison. Tropical climates are usually stated to cause alterations in the blood, and if this is true European standards are not applicable to tropical medicine without test of validity. Few investigations appear to have been made on this important subject. Forty-three text-books of tropical medicine, European medicine, physiology, etc. gave figures, but none to which statistical tests had been applied; and the majority gave normal ranges far narrower than would be expected, assuming that differential counts behave like the majority of variable populations. Comparison was complicated by differences in the names of the mononuclears; allowing for this, a résumé of the data is given in Table I.

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Only KILDUFFE (1931) and TIDY (1934) gave the range of polymorphs as 50 to 75 per cent., none gave extreme ranges of eosinophils, lymphocytes, and monocytes so wide as the figures above, which are taken over all the texts consulted. Recent studies in Iraq showed that the polynuclear count and differential count did not conform to these standards (KENNEDY, 1935; KENNEDY and MACKAY, 1935 and 1936), and it was felt that the importance of the leucocyte picture in clinical practice justified the extension of the work. Under tropical conditions opportunities are infrequent for studying the physiological states of large groups of healthy Europeans owing to the relatively small size of the populations, the calls of routine work on the practitioner, and it may be added that the summer heat tends to induce an irritability of temper or a *laisser faire* attitude which is not conducive to useful co-operation.

MATERIAL AND METHODS.

KENNEDY and MACKAY (1936) studied the leucocyte picture in 271 airmen and officers of the Royal Air Force (177 stationed at Hinaidi, 32 at Amman, Transjordania, 33 at Aden, and 29 at Khartoum, Sudan) and 29 Iraqi medical students. To the results are now added those from 30 airmen stationed at Shaiba, Iraq, 49 more stationed at Hinaidi, and 21 Iraqi medical students, bringing the total to 400. As it was pointed out before that the Royal Air Force population offered special advantages for the investigation, the most important being that the health standard was singularly high, it is not necessary to recapitulate the detailed reasons. The Iraqi students were drawn from a socially favoured class, had undergone a medical examination before entering College, and were re-examined to exclude minor pathological influences. At the same time parallel investigations were made on pathological groups to discover the statistical differences from the normal standard, and to find whether the polynuclear count reacted to diseased conditions as it does in Europe.

Malaria.—The clinical material in this group was not so uniform as in the case of the normals. Forty-six were British airmen, and 254 were Iraqis of all social classes with an age group of 12 to 59, though the majority were Iraq Levy troops between 19 and 26. The samples were mostly collected at the height of the malaria season, but some were taken throughout the year. Of a total 300 cases, 171 were benign tertian, 122 were malignant, and only 7 were quartan, the last being comparatively rare in Iraq. The severity of the infections varied within wide limits, but in all cases parasites were observed in the bloods examined. It has been shown that the blood reaction varies with the different stages of the parasite's development (GARIN, 1930), further some of the cases were new infections, a few had received quinine, and some were relapses. Thus comparison with the normal group is only to be made with considerable reservation. It must be emphasized that the aim of this part of the research was to find the range of relative leucocyte reaction which could occur in malaria and the other pathological conditions, rather than to study the detailed response in groups

where as many variables as possible were controlled. Such observations would have required an impracticable number of cases, and would have introduced complications unnecessary at this stage. On the other hand it is possible that the diversity of sampling did not introduce such a disturbance of the basic statistics as might be expected, since it has been shown that so far as the polynuclear index was concerned, a male Iraqi population, healthy but otherwise taken at random, did not differ statistically from one of the airmen (KENNEDY and MACKAY, 1935).

Tuberculosis.—This group included 97 cases of active phthisis of whom two were British airmen, the rest being young Iraqi adults, and 36 cases of surgical tuberculosis. Again, it must be made clear that the inclusion of two different types of tuberculosis in the same division is permissible only since a general view of the blood reaction to the disease is wanted.

Leprosy.—The lepers were all of the nodular type, 72 being from Baghdad, 32 from Amara, and 29 from Basrah. All were Arab fellahin, and only 10 were women.

Phlebotomus or Sand-fly Fever (three-day fever).—Of these cases 101 were British airmen and 29 were Assyrians of the Iraq Levies. Difficulty is also encountered in this group for the differential diagnosis of phlebotomus fever, in Iraq at least, is not entirely satisfactory. Similar brief fevers are not uncommon which, however, differ sufficiently from sand-fly fever to suggest that the ætiology is different.

Bilharziasis.—The 100 cases were infections with *Schistosoma haematobium* drawn from all ages of the male population of a village, Han-beni-Saad, near Baghdad, where the disease was endemic. The diagnosis was made from the presence of ova in the urine. The samples were taken during an intensive treatment campaign, and opportunity was unfortunately not then afforded to examine the stools for helminthic infestation. It is probable that a proportion, estimated at 5 to 10 per cent. may also have been infected with ankylostomiasis. *Ascaris* and *Taenia* are not common in this district.

The methods of preparation and counting were the same as detailed in the previous papers, and it will suffice to discuss the classification of the leucocytes. A feature of both normal and pathological groups was the relatively large number of cells with ruptured membranes and scattered granules, and degenerate cells of the smear and basket types. Eosinophils were especially fragile. The degenerate cells were omitted from the count, but eosinophils, which only appeared to have ruptured through drawing the film, were included.

The division of granulocytes into neutrophil, eosinophil, and basophil is familiar and needs no remark. Opinions differ regarding non-granular cells. The lymphocytes are divided by certain writers into large and small; the former are generally considered immature, as shown by the more constant presence of nucleoli; and are relatively more common in children. All stages of intermediate forms occur, so the division into two groups is arbitrary and unnecessarily

complicates the differential count. Further, there does not seem to be any well-founded clinical reason for making a separation.

The upholders of different theories of the origin of monocytes have given these cells a number of names, among which are large uninuclears, large mononuclears, large hyalines, macrocytes, splenocytes, endotheliocytes, endothelial leucocytes, and transitional cells. The last was applied on the incorrect assumption that there was a transition form between the monocyte and the neutrophil. MAXIMOW and BLOOM (1935) state that in most animals it is impossible to separate all monocytes as distinct from lymphocytes, and that transitions between them are usually present. In the present investigation cells occurred which were difficult to classify as either monocytes or lymphocytes and appeared to be intermediate types. No special class was made for these, but they were included in the group they most resembled. Different forms of monocytes can be distinguished but there does not appear to be any advantage in separating them. All cells conforming to the following description were considered monocytes. The diameter is usually between 16 and 22μ, though the upper limit may be exceeded. The nucleus is relatively smaller than that of the lymphocyte, and is eccentrically placed, round or oval (but if so, the margin is always slightly indented), reniform, horseshoe shaped, or deeply constricted. The nucleus stains paler than that of the lymphocyte; the nuclear membrane is thinner. The chromatin granules are finer and more numerous, the appearance is generally of a loose mesh with thickenings where the chromatin threads meet. The cytoplasm is a pale greyish-blue colour, sometimes pale violet, and with critical illumination well-stained slides show reddish-blue granules much finer and more numerous than those of the neutrophil. In the pathological series particularly, monocytes were found with polymorph nuclei showing three, four or even more lobes, the staining reaction being always that of the typical monocyte. As many of this type had vacuolated cytoplasm, it is considered that they were senile or degenerating from pathological causes.

The occurrence of cells other than the above in normal blood is seldom mentioned. DIEULAFOY (1912), however, says certain rare or abnormal elements occur, not exceeding 1 to 2 per cent. These he gives as plasma cells, mononuclear basophils, myelocytes and maztzellen. OSGOOD and HASKINS (1931) define metamyelocytes, or cells intermediate between staff cells (Stabkernige) and myelocytes thus:—"any cell with specific granulations in the cytoplasm which has a nucleus which is not segmented and cannot be described as either round or oval, nor as a curved or coiled band, should be classed as a metamyelocyte." It is said that metamyelocytes occur rarely in normal blood, while NICHOLSON (1934) states there is less than 1 per cent. in the blood of normal people. GRADWOHL (1935) agrees that they are found very occasionally in a percentage of 0 to 1. OSGOOD and HASKINS (1931) criteria for distinguishing these, and other unusual cells, have been adopted here as they are clear, distinct and perhaps the least liable to cause confusion.

The myelocyte is a cell with a round or oval nucleus which does not contain nucleoli, and has neutrophilic or very slightly basophilic cytoplasm containing specific granulations, but not azur granules. No myelocytes were found in the normal group.

Promyelocytes are intermediate in structure between stem cells or myeloblasts, and myelocytes.

Stem cells, myeloblasts, or lymphoblasts have deeply basophilic cytoplasm, and a relatively large round oval nucleus containing nucleoli, which though finely reticular is almost homogeneous. Azur granules may occur in the cytoplasm. The distinction between myeloblast and lymphoblast depends on criteria so slight as to be unreliable, though in pathological bloods the predominant features of the differential count often make it possible to place a cell in one of the two groups. PINEY (1931) and also GRADWOHL (1935) describe a monoblastic type of stem cell, and while some specimens were found which resembled their descriptions in certain respects, none fitted them exactly. It has been thought best to include all these under the term stem cells, particularly as they only occurred sporadically.

The Rieder cell resembles the stem cell except that the nucleus is a coiled or curved rod or band, though its internal structure is that of the myeloblast. The ones found here were all smaller than stem cells.

Plasma cells have an eccentric nucleus and a large amount of very basophilic cytoplasm, the circumnuclear area being paler than the remainder. They have little diagnostic value, but are sometimes found in the course of infections which cause a lymphocytosis. None occurred in any normal blood of this series. The Türk irritation form has the cytoplasm of the plasma cell, is often vacuolated, while the nucleus resembled that of the stem cell. Some authorities believe they are identical with the plasma cell. They occur in many conditions in which there is assumed to be an irritation of the bone marrow, but are not considered of diagnostic significance.

The basis and methods of the polynuclear count have been fully discussed in the two previous papers in these TRANSACTIONS. It will suffice to say it gives an index of mean maturity of the neutrophilis.

RESULTS.

The principal statistics of the normal group are given in Table II. As graphic comparison is often more vivid than numerical, frequency histograms of this and the pathological groups are shown in the diagram on p. 314. In the figure the pathological sets have been stepped up to the same percentage scale as the larger normal group to facilitate comparison. As the numbers of basophils and " abnormal " cells is so small, standard deviations, etc., have not been calculated for them but a detailed analysis is set out in Table VII (p. 319).

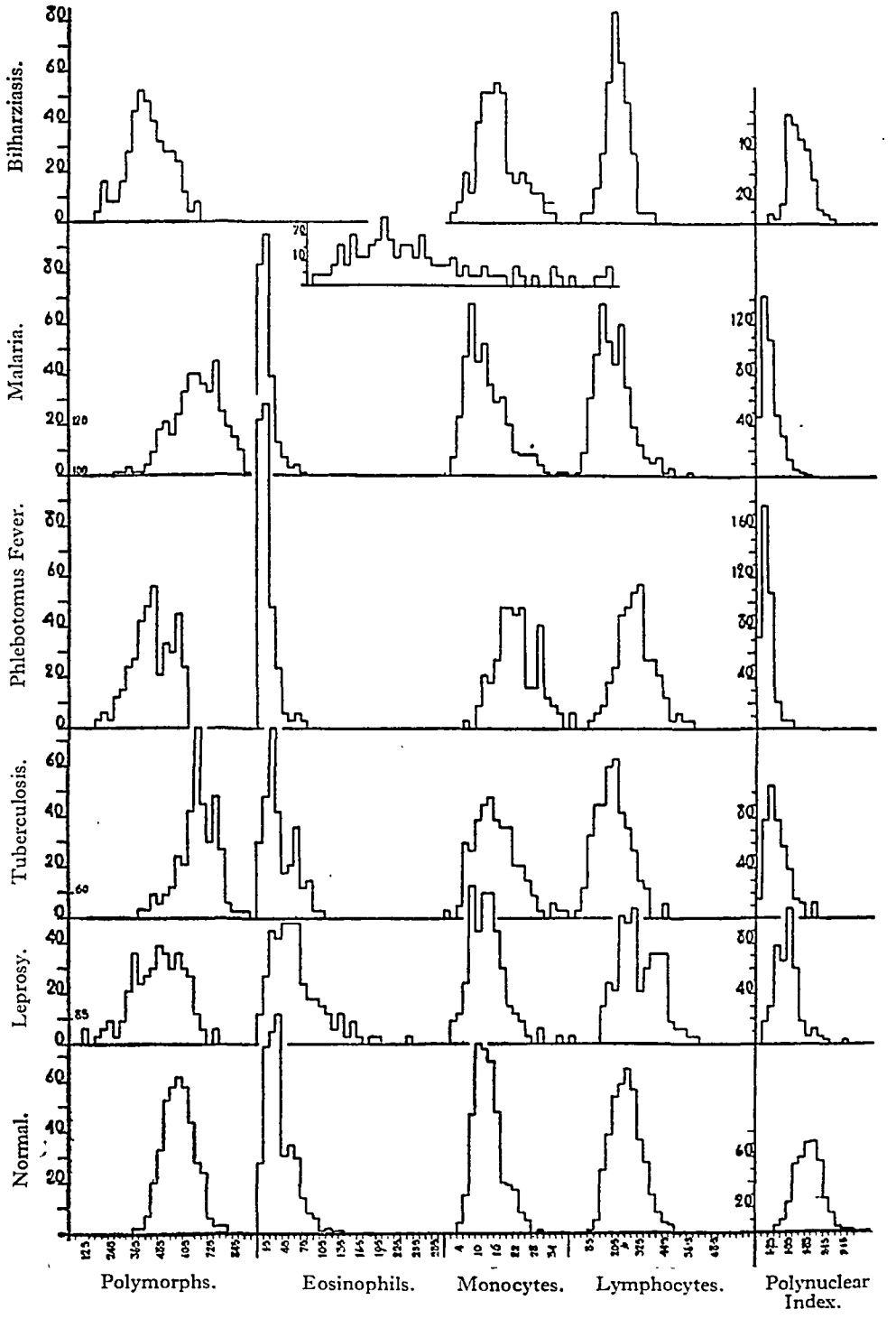

Bilharziasis.
Malaria.
Phlebotomus Fever.
Tuberculosis.
Leprosy.
Normal.
Polymorphs.
Eosinophils.
Monocytes.
Lymphocytes.
Polynuclear Index.

TABLE II.
400 NORMAL INDIVIDUALS.

	Mean.	Standard Deviation.	Standard Error. ±	Coefficient of Variation.	Range.	
					Low.	High.
Polymorphs	57·10	7·23	0·363	12·66	35·5	75·0
Eosinophils	3·63	2·67	0·113	62·49	0	13·0
Lymphocytes	26·07	7·25	0·363	27·87	9·0	48·0
Monocytes	13·08	4·57	0·228	34·93	3·0	29·5
Polynuclear index	1·864	0·206	0·103	11·08	1·30	2·51

It will be seen that the mean percentage of the different cells does not add up exactly to 100. This is due to the method of grouping the data. By centering a group at the middle value of its range of x (instead of its mean value which would be ideal, but practically laborious) a certain error is introduced, and it is this error (which in practice is nearly negligible) which has accumulated over the averages of the different cell groups and produced the discrepancy. The danger of the statistical method is to strain after greater accuracy than the conditions of the experiment warrant, and while it would be possible to extract means for these percentages which would total 100, the results would not justify the labour necessary, and the additional fineness would not be consistent with the method of the observations.

Compare the normal polymorph data with those in Table I. The mean is 10·9 per cent. less, and the range is 15 per cent. greater than the extreme range which, it will be remembered, was only given by two authorities out of forty-three. The question which immediately appears is whether the present results constitute a probable or an improbable event. Characters of not excessively small probability when sampled in a large number of trials tend to normal distribution under a wide variety of circumstances. Statistical tests were therefore applied to discover the type of curve given by the experimental results. Following the method of ELDERTON (1927) the curve criteria of PEARSON'S system of frequency curves were calculated, then by means of his table it was decided which curve should be used.

TABLE III.
CURVE CRITERIA OF DISTRIBUTIONS IN NORMAL GROUP.

	β_1	β_2	κ
Polymorphs	0·00001732	2·6671	−0·00001958
Lymphocytes	0·13806	2·8760	−0·15656
Monocytes	0·5684	3·4592	−0·89936
Eosinophils	1·2185	4·4476	−1·6944
Polynuclear index	0·001628	2·8520	−0·004071

The polymorphs and lymphocytes satisfy the criteria for the Normal or Gaussian curve fairly well, that is $\beta_1 = 0$. $\beta_2 = 3$. $\kappa = 0$. The next step was to fit a normal curve to the distribution and examine the agreement by means of the χ^2 test. The area method of fitting the normal curve was used (ARKIN and COLTON, 1935). The details are set out in Table IV. The data in column 6 were derived from the table given by PEARL (1930).

TABLE IV.

FITTING A NORMAL CURVE TO THE POLYMORPH DISTRIBUTION.

Class limits.	Class mid points.	Frequency of class f	Deviation of class limit from mean x	Deviation in terms of class interval x/σ	Per cent. area between class limit and mean	Per cent. area in class interval.	Theoretical frequency ft
35–37·99	36·5	2	22·3	3·08	49·90	0·28	1·1
38–40·99	39·5	2	19·3	2·67	49·62	0·84	3·4
41–43·99	42·5	7	16·3	2·25	48·78	2·07	8·3
44–46·99	45·5	20	13·3	1·84	46·71	4·49	18·0
47–49·99	48·5	33	10·3	1·42	42·22	7·84	31·4
50–52·99	51·5	53	7·3	1·01	34·38	12·14	48·6
53–55·99	54·5	58	4·3	0·59	22·24	15·10	60·4
56–58·99	57·5	62	1·3 1·7	0·18 0·23	7·14 9·10	16·24	65·0
59–61·99	60·5	58	4·7	0·65	24·22	15·12	60·5
62–64·99	63·5	44	7·7	1·06	35·54	11·32	45·3
65–67·99	66·5	28	10·7	1·48	43·06	7·52	30·1
68–70·99	69·5	24	13·7	1·89	47·06	4·00	16·0
71–73·99	72·5	6	16·7	2·31	48·96	1·90	7·6
74–76·99	75·5	3	19·7	2·72	49·67	0·71	2·8

FISHER (1932) states, " For any value of n, which must be a whole number, the form of distribution of χ^2 was established by PEARSON in 1900 ; it is therefore possible to calculate in what proportion of cases any value of χ^2 will be exceeded. This proportion is represented by P, which is therefore the probability that χ^2 shall exceed any specified value. To every value of χ^2 there thus corresponds a certain value of P ; as χ^2 is increased from O to infinity, P diminishes from 1 to 0. Equally, to any value of P in this range there corresponds a certain value of χ^2. Algebraically the relation between these two quantities is a complex one, so that it is necessary to have a table of corresponding values if the χ^2 test is to be available for practical use."

In applying the Pχ^2, the small frequencies at the ends of the distribution were pooled into single larger classes. The use of χ^2 rests on a mathematical approximation which is satisfactory if class frequencies exceed 10 or 12, but is somewhat in error if any very small frequencies are present. Further, since the mean and standard deviation were not given *a priori*, but were computed from

the data, it was necessary to allow for this amount of forced agreement by subtracting what FISHER calls " degrees of freedom," that is, to find, n the point at which his table of $P\chi^2$ is entered, 2 is subtracted from the number of classes (one for the mean, and one for the standard deviation). The method of determining χ^2 is shown in Table V.

TABLE V.

" GOODNESS OF FIT "—DATA OF TABLE IV.

Class mid point.	Observed frequency f	Theoretical frequency f_t	$(f - f_t)$	$(f - f_t)^2$	$\frac{(f - f_t)^2}{f_t}$
36·5	2	1·1			
39·5	2	3·4	−1·8	3·24	0·253
42·5	7	8·3			
45·5	20	18·0	2·0	4·00	0·222
48·5	33	31·4	1·6	2·56	0·082
51·5	53	48·6	4·4	19·36	0·398
54·5	58	60·4	−2·4	5·76	0·095
57·5	62	65·0	−3·0	9·0	0·139
60·5	58	60·5	−2·5	6·25	0·103
63·5	44	45·3	−1·3	1·69	0·037
66·5	28	30·1	−2·1	4·41	0·147
69·5	24	16·0	8·0	64·0	4·000
72·5	6	7·6	−1·4	1·96	0·188
75·5	3	2·8			
					$\chi^2 = 5·664$

Using FISHER'S (1932) table we find that for $n = 9$ and $\chi^2 = 5·664$, that P lies between 0·8 and 0·7, and by interpolation the value is approximately 0·77. This means that if the frequencies were normally distributed, we should expect to obtain a worse fit by a normal curve in approximately 77 per cent. of future samples of 400 cases. Hence for a sample of 400 the observed deviations from the normal curve are in no way exceptional, and we conclude that the distribution of frequencies is adequately represented by a normal curve.

In similar fashion the theoretical normal curve was constructed for the polynuclear index, the χ^2 test applied, and P was found to be 0·84, that is, a satisfactory fit. Likewise the data for lymphocytes was similarly treated, and P was 0·59 which is rather less good owing to the presence of slight positive skewness, but still may be considered a satisfactory fit. The monocytes and eosinophils appear to satisfy the criteria for PEARSON'S Type I curve rather than any other, but this point was not pursued since it did not seem that any conclusion that might be drawn would be immediately useful. It would not be expected that these two distributions would be of the " normal " type for

they form a relatively small proportion of every hundred cells examined, and the means are relatively small numbers.

The number of basophils found was so small that no distributions were attempted. The statistics are given in Table VI, and those for the pathological material are included for the sake of brevity.

TABLE VI.

OCCURRENCE OF BASOPHILS.

	Normal.	Malaria.	Leprosy.	Tuberculosis.	Phlebotomus Fever.	Bilharziasis.
Number of cases	400	300	133	133	130	100
Number with basophils	35	52	63	31	9	53
Percentage with basophils	8·8	17·3	47·4	23·3	6·9	53
Average per case	0·067	0·102	0·775	0·169	0·055	0·460
Range, 0 to	2·5	1	3	1·5	1·5	2

In like manner the occurrence of so-called " abnormal " cells is set out in Table VII. The figures in the first column are the percentages found for each kind of cell, and the corresponding figures in the subsequent columns give the total number of individuals in which each percentage occurred.

One case might have 1·5 per cent. of metamyelocytes, 0·5 per cent. of stem cells, and say, 0·5 Rieder cells. This overlapping could not be readily shown, but the total effect can be gauged by inspecting the second and third lines of the Table.

In Table VIII the statistics of the pathological material have been collected. To facilitate comparison the figures for the healthy population have been included, and the grouping has been made primarily in cell types rather than diseases. The mean and standard deviation require no explanation. The Standard Error of the mean has been used in preference to the Probable Error, following FISHER (1932) who says : " The common use of the probable error is its only recommendation ; when any critical test is required the deviation must be expressed in terms of the standard error." If instead of making one series of N observations, a large number, m, of a series of means were obtained, each series having the same number of N observations, the m means would be distributed in a normal curve, the standard deviation of which is $\sigma/\sqrt{N} =$ the standard error of the mean. Since 99·73 per cent. of cases in this universe will be included within a distance of 3 standard deviations from the mean, then in 99·73 chances out of 100 no error (difference between the sample mean and " true " mean) will occur larger than 3 times the standard error. The coefficient of variation, $v = 100\frac{\sigma}{M}$ or the percentage ratio of the standard deviation to the arithmetic mean, was

TABLE VII.
OCCURRENCE OF SO-CALLED "ABNORMAL" CELLS.

	Percentages.	Normal	Malaria.	Leprosy.	Tuberculosis.	Phlebotomus Fever.	Bilharziasis.
Number of cases		400	300	133	133	130	100
Total with abnormal cells		242	237	72	81	110	51
Percentage with abnormal cells		60·5	79	54·2	62·3	84·6	51
Metamyelocytes	0·5	107	63	23	19	28	13
	1·0	57	26	8	9	24	2
	1·5	19	21	3	2	16	
	2·0	4	15	2	0	8	
	2·5	3	6	0	1	2	
	3·0	2	8	0		1	
	3·5	1	2	1			
	4·0	2	2				
	4·5	1	0				
	5·0		0				
	5·5		0				
	6·0		1				
	6·5		1				
Myelocytes	0·5	—	19	8	6	8	1
	1·0		1	1	1		
	1·5		2				
	2·0		2				
	2·5		1				
Promyelocytes	0·5	6	8	1	—	2	1
	1·0	6	3				
Eosinophil-meta-myeolocytes	0·5	11	1	12	3	3	5
	1·0			0			6
	1·5			1			1
Eosinophil-myelocytes	0·5	4	1	2	1	—	2
	1·0	2					
Basophil-metamyelo-cytes	0·5	—	—	—	1	—	
Macropolycytes	0·5	1	10	7	8	1	—
Stem cells	0·5	34	67	16	27	15	8
	1·0	5	20	1	6	6	
	1·5		9		1	2	
	2·0		6		2	0	
	2·5		0			1	
	3·0		1				
Plasma cells	0·5	—	13	7	8	1	3
	1·0		12	0	5		2
	1·5		1	1			0
	2·0		1				1
	2·5		0				
	3·0		0				
	3·5		1				1
	4·0		0				
	4·5		0				
	5·0		0				
	5·5		0				
	6·0		1				
Rieder cells	0·5	2	3	—	1	—	4
	1·0				1		2
Türk cell	0·5	3	25	16	11	9	11
	1·0		17		3	3	3
	1·5		3		1		
	2·0		4		1		
	2·5		2				
	3·0		1				
	..		0				
	5·5		1				
	..		0				
	8·5		1				

devised by PEARSON (quoted from YULE, 1932) as a measure of relative dispersion. It is a mere number and is independent of the units of measurement employed, but is useful in the comparison of the amount of dispersion in differing groups.

The curve criteria for the pathological data have not been calculated since the groups are much smaller than the healthy population, too many uncontrolled variables enter into them, and the information derived from classifying these distributions would have little medical importance. An inspection of the histograms on p. 314 shows most are more skew than the corresponding normals but with the exception of the eosinophils in bilharziasis, they do not depart from the " expectation of distribution." These eosinophils have a very wide range so that the number of classes on the same scale as the others is large while the number of cases in each class is relatively small. It is probable that if, say, 1,000 cases had been examined, the distribution of the population would approximate to " normal " though slight skewness might occur.

The data in Table VIII enables us to discover if the groups differ significantly from each other, that is, whether the observed differences are small enough to be within the limits of sampling error or whether they are so great that the event of their being determined by chance is highly improbable. In the present instance the only important differences are between each of the pathological groups and the healthy group. The standard error of the difference of two means (standard deviation of the theoretical distribution of differences between means of samples) can be obtained from the equation :—

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$$

Where

σ_1 = standard deviation of the first sample.
σ_2 = standard deviation of the second sample.
N_1 = number of items in first sample.
N_2 = number of items in second sample.

If the difference between the means is greater than $3\times$ the standard error of the difference between the means (that is, if the probability of such a difference being due to chance is very small) one can say the difference is significant and not due to chance. The results of the application of this test are given in Table IX. The columns headed " Difference " refer to the difference between the mean in each case and that of the healthy population. The difference is not significant in four instances only; the eosinophils in tuberculosis, the lymphocytes in phlebotomus fever, and the monocytes in malaria and leprosy.

DISCUSSION.

The analysis of the results of the differential counts is consistent with the general expectation of the distributions of biological commensurables, and it may be concluded that the samples are fair representatives of the particular

TABLE VIII.

STATISTICS OF PATHOLOGICAL MATERIAL.

		Mean.	Standard Error. ±	Standard Deviation.	Coefficient of variation.	Range.	
						Low.	High.
Polymorphs	Normal	57·10	0·363	7·23	12·66	35	75
	Malaria	66·18	0·628	10·88	16·44	26	90
	Tubercle	68·10	0·843	9·72	14·28	38	93
	Leprosy	47·87	1·055	12·16	25·41	12	75
	Phlebotomus fever	44·92	0·860	9·79	21·80	18	60·5
	Bilharziasis	42·98	1·004	10·44	24·32	19·5	67·5
Polynuclear index	Normal	1·864	0·0103	0·206	11·08	1·30	2·50
	Malaria	1·207	0·0079	0·137	11·36	1·04	1·78
	Tubercle	1·342	0·0151	0·174	12·95	1·03	1·96
	Leprosy	1·513	0·0184	0·213	14·10	1·13	2·44
	Phlebotomus fever	1·188	0·0089	0·101	8·50	1·04	1·57
	Bilharziasis	1·700	0·0188	0·188	11·07	1·23	2·22
Eosinophils	Normal	3·63	0·113	2·67	62·49	0	13
	Malaria	1·99	0·635	1·10	55·20	0	7
	Tubercle	4·27	0·244	2·82	65·91	0	10·5
	Leprosy	6·54	0·360	4·15	63·41	0	25
	Phlebotomus fever	1·46	0·010	1·18	81·10	0	7
	Bilharziasis	25·89	1·002	10·02	38·72	9·5	56·5
Lymphocytes	Normal	26·07	0·363	7·26	27·87	9	48
	Malaria	19·70	0·492	8·51	43·23	3	56
	Tubercle	19·51	0·702	8·08	41·48	3	45
	Leprosy	31·98	0·866	9·97	31·34	13	58
	Phlebotomus fever	28·12	0·837	9·54	33·96	4·5	57
	Bilharziasis	23·18	0·645	6·45	27·84	10	40
Monocytes	Normal	13·08	0·228	4·57	34·93	3	29·5
	Malaria	12·58	0·379	6·55	52·08	1	38
	Tubercle	9·24	0·311	3·59	38·86	1	20
	Leprosy	13·21	0·511	5·89	44·59	2	39
	Phlebotomus fever	22·34	0·579	6·59	29·54	5·5	39·5
	Bilharziasis	8·56	0·331	3·31	38·66	1	17

TABLE IX.

SIGNIFICANCE OF MEANS COMPARED WITH NORMAL STANDARD.

	Polymorphs.		Eosinophils.		Lymphocytes.		Monocytes.		Polynuclear index.	
	Difference.	$3\sigma_D$	Difference.	$3\sigma_D$	Difference.	$3\sigma_D$	Difference.	$3\sigma_D$	Difference.	$3\sigma_D$
Malaria	9·08	2·173	1·64	0·443	6·37	1·834	0·50	1·324*	0·657	0·0389
Tuberculosis	11·00	2·761	0·64	0·836*	7·19	2·368	3·84	1·157	0·512	0·0580
Leprosy	9·23	3·350	2·91	1·060	5·91	2·814	0·13	1·678*	0·351	0·0634
Phlebotomus fever	12·18	2·800	2·17	0·505	2·05	2·738*	9·26	1·864	0·676	0·0406
Bilharziasis	14·12	3·320	22·16	1·960	2·89	2·220	4·52	1·205	0·164	0·0643

* Difference not significant.

universes. The percentage count is divided into unequal classes, three (neutrophils, lymphocytes and monocytes) being considerably larger than the others. The larger classes would be expected to approximate to the normal curve, though probably with some degree of skewness. The smaller classes would not be expected to fit the normal curve as the means are under 10. The histograms of the normal neutrophils and lymphocytes are slightly platykurtic, that is, β_2 is less than 3, being 2·667 and 2·867 respectively. The difference is not statistically significant. The standard error of the kurtosis is :—

$$\sigma_{\beta_2} = \sqrt{\frac{24}{N}} = 0{\cdot}245$$

and $3\sigma_{\beta_2}$ is greater than the actual difference. The normal monocytes are slightly, but not significantly, leptokurtic, β_2 being 3·459, but the curve is also more skew. The measure of the skewness suggested by PEARSON is the distance between the mean and the mode divided by the standard deviation, or in terms of moments

$$\chi = \frac{\sqrt{\beta_1}\,(\beta_2 + 3)}{2\,(5\beta_2 - 6\beta_1 - 9)}$$

The larger the value of χ, the greater is the departure of the curve from symmetry. Applying this equation to the values given in Table III, we have

for normal neutrophils $\chi = 0{\cdot}0833$
for normal monocytes $\chi = 0{\cdot}4984$

Of the normal group the monocytes differ most from the text-book standard. When at first some very high monocyte counts were found the results were viewed with suspicion, the most obvious possibility of error was that some lymphocytes were being classed as monocytes. Thereafter great care was taken to avoid this, but no difference resulted. It has been noted that degenerate cells were more commonly encountered here than in Britain, and that cells occurred which were difficult to classify as between lymphocytes and monocytes. Both factors required careful attention, and it is emphasised that when doubt arose all criteria of each class were considered before a decision was made. While the average monocyte range of the text-books is from 3·8 to 6·8, MARTINET (1934) gives a range of 12 to 15, CHIRAY and CHENE (1934) from 10 to 12, and NICHOLSON (1934), WHITBY and BRITAIN (1935), and WOOD (1918) give ranges of 5 to 10 per cent. In view of these figures, and the frequent statement that monocytes tend to increase in the tropics (though we have not seen the extent of the increase mentioned) the mean of 13·08 per cent. found here does not appear so unlikely. Further, in a sample of 400 values normally distributed about a mean of 13, a few values exceeding 25 would be expected. After careful consideration it has therefore been concluded that the data for the monocytes give a true picture of the sample, and have not been significantly disturbed by avoidable errors.

Similar doubts were felt as to the validity of such high counts as over

10 per cent. eosinophils. The cases in which these occurred were symptomless, and negative to repeated tests for worm infection. POINDEXTER (1935) has reported on 401 apparently healthy negroes in Alabama who were negative for malarial parasites, helminthic parasites in the stools, tuberculin reaction; and who had no history of any disease usually associated with eosinophilia. He found an average of 8·3 per cent. eosinophils over all, and in the age groups 5 to 9 and 15 to 19, percentages of 12·7 and 10·3 respectively. PETERSEN (1936) reports high eosinophil counts from Hawaii. STITT (1929) refers to NEISSER'S belief that increased eosinophil may be an expression of sympathetic system irritation; and there is some evidence that the latter is a factor worth considering in Iraq. Further, a symptomless familial eosinophilia is well recognized, though reports are few. Again, the figures may be accepted as a true reflection of the facts.

Relatively high numbers of the so-called " abnormal cells " were observed. Desultory references to the occurrence of these in normal blood has been made by DIEULAFOY (1912) and other authors, though they are more frequently passed over. Metamyelocytes constituted the bulk of the unusual types in the normal group, the next most numerous being stem cells. Both tend to appear when the marrow is stimulated to extra activity and the evidence afforded by the polynuclear count indicates that this stimulation does in fact take place. Macropolycytes are dealt with fully by KENNEDY and MACKAY (1937).

The normal percentage of basophils is usually given as 0 to 1, with an average of 0·5, but the proportion of people in whom they are absent is only mentioned by COOKE (1928) (*ca.* 60 per cent.). In the course of 11 years' teaching histology in Edinburgh to classes of over 200 students difficulty was always experienced in finding basophils for demonstration in the students' blood films, and though no records were kept, the impression is that they occurred in about 10 per cent. of the films. The incidence of basophils in 8·8 per cent. of normals here cannot be regarded as unusual.

The distribution of the polynuclear counts fits the normal curve well and it can be accepted as a true representation of the facts. As the basis of the count, and its special relation to Iraq have been discussed in the previous papers, this need not be repeated here.

Data from a similar group of healthy Britons were not available for statistical comparison but the consensus of opinion in standard text-books makes it reasonable to conclude that there is an alteration of the leucocyte picture in this country, though it should be added that other recent work tends to throw doubt on the validity of accepted standard. OSGOOD and HASKINS (1931) in Oregon are of the opinion that the neutrophil range of 55 to 75 per cent. (average 64 per cent.) is from 5 to 15 per cent. too high and the figures for lymphocytes are correspondingly too low. They are certain that neutrophil counts over 70 per cent. must be interpreted with caution. It is, however, generally agreed, that blood cell changes occur in the tropics, though more attention has been paid to erythrocytic differences than to leucocytic.

Observations have been made indicating that alterations of geographical locale other than tropical apparently influence the blood picture. GÄNSSLEN (1937) has recently reported regional differences in leucocyte counts in patients suffering from other than blood diseases in Tübingen and Frankfort, and notes that he, and others who moved from Tübingen to Frankfort, underwent profound leucocytic changes. The total counts fell; the percentage of lymphocytes approximately doubled. He concludes that the leucocyte count must be influenced by geographical and climatological factors, though the precise nature is yet unknown.

STAMMERS (1935) examined 171 healthy European adults in Johannesburgh, 5,750 feet above sea level, and compared the blood picture with the means of 68·2 per cent. neutrophils and 25·8 per cent. lymphocytes, which were derived from twelve standard texts. His results are given (a) in Table X which includes also his quotation from SACHS on 81 natives at Pretoria Mental Hospital (b).

TABLE X.

	Mean.	Standard Error. ±	Standard Deviation.	Range.
(a) Neutrophils	54·2	0·510	6·670	38·0–73·0
Eosinophils	1·88	0·094	1·231	0·2– 7·0
Basophils	0·69	0·042	0·548	0·0– 2·6
Lymphocytes	39·72	0·473	6·190	21·0–55·3
Monocytes and transitional	4·24	0·097	1·272	1·7– 7·5
(b) Neutrophils	54·70	0·247	3·23	38·0–72·0
Lymphocytes	36·80	0·578	7·56	21·0–53·0

The most striking differences between these figures and those in Table II are for the lymphocytes, which are much higher here, and the monocytes, which are much less.

Several papers have reported deviations of the polynuclear count from COOKE and PONDER'S (1927) standard in various localities. Among them MACLEOD gives figures for eleven widely scattered points from Alberta to Australia; others are BURKE-GAFFNEY (1931) (Tanganyika); SHAW (1935) (Egypt); SHANKLIN (1936) (Syria); PAI (1936) (China), who also quotes BANNERJEE (India); ABELS (1934) (New York); similar deviations using ARNETH'S (1904) method are recorded by DUKE (1920) (Uganda); BREINL and PRIESTLY (1934), Australia and New Guinea, quoted by PAI (1936); CHAMBERLAIN and VEDDER (1911) (Philippine Islands); MACFIE (1916) (Gold Coast, quoted by SHAW). Significantly different counts have been obtained in these localities, but the distributions about the respective means show that the index is remarkably constant for each locality. Thus the validity of the method as a sensitive test of the neutrophil state is not impaired. Before it can be used clinically it is necessary to establish a local standard of reference.

After considering etiological factors which might be responsible for the alteration of the blood picture of British Airmen in Iraq, KENNEDY and MACKAY (1935 and 1936) concluded the likeliest agent was climate, which is in line with the opinions of most writers. One would not claim this is the only factor, but a circumspect examination of the airmen's food, habits, exercise, health and general environment failed to disclose any other agency on which responsibility could be placed.

Space does not permit a detailed discussion of the influence of climate, which will be made the subject of a further study dealing with variations in temperature, air density, ultra-violet radiation, etc.

Pathological Material.

This was less uniform than the normal group. No attempt was made, for example, to subdivide the cases according to severity of infection. The aim was to find the range of deviation which could occur in the leucocyte picture which might be met clinically in Iraq in any instance of the particular conditions. This is perhaps a somewhat crude criterion but in the absence of established figures it had to be applied before more stringent classifications could be used.

Malaria.

The usual teaching is that a monocytosis accompanies malaria. WHITBY and BRITTON (1935) say an increase of the monocytes to the order of 15 per cent. is of definite diagnostic value. The present results are at first sight contradictory to this for all the means of cell types in malaria differ significantly from the normal standards *except* that of the monocytes. The neutrophils are increased, the lymphocytes and eosinophils diminished. But the coefficient of variation of the monocytes is greater (52·08 against 34·93), as also is the range (1–38 against 3–29·5). If the results were compared with the text-book percentage for monocytes there would apparently be monocytosis. In the absence of data for a large control group of normal persons in some tropical locality, a practitioner there, never having occasion to examine normal bloods, might well be misled by the malarial differential counts into assuming the existence of a relative monocytosis. Further, some of the cases showed very high neutrophil percentages—up to 90. This would reduce the monocyte percentage, and lower the mean value over the series.

The neutrophils may vary within wide limits as there is a leucocytosis during the paroxysm and a leucopenia in the apyrexial stage. GARIN (1930) found a massive increase of neutrophils prior to the paroxysm which diminished progressively during the attack, and in 72 cases there was a left deviation of the Arneth count.

In the present instance 117 of the 300 cases showed no eosinophils. A high proportion of abnormal cells occurred, including notably myelocytes, macropolycytes, stem cells, Türk cells, and plasma cells.

When the sample population is drawn from a universe of malarial patients at all stages of the disease, the common factor being the presence of the parasite in the peripheral blood, a typical blood picture cannot be defined. It is possible, however, to say that for Iraq at least, monocytosis cannot be taken as a diagnostic character of malaria.

Tuberculosis.

Considerable variation in the blood picture may be found in the different kinds of tuberculosis. A low total count with a relative lymphocytosis is generally considered typical, and a leucocytosis combined with a marked " shift to the left " is taken as indicative of secondary infection. In the acute miliary form there may be extreme leucopenia with the majority of the cells neutrophils ; in less acute degrees there may be a neutrophil leucocytosis. The chronic pulmonary type in healing shows progressive changes from neutrophilic to monocytic to lymphocytic predominance. Several authors state that monocytes are increased. MEDLAR (1929) says there is no specific haemacytic reaction in the tuberculous, and that four variables are to be considered. First, the neutrophil plays the chief role in tuberculous abscess formation and in the extension of tuberculous ulcers ; second, lymphocytes predominate when the lesions are healing ; third, the monocyte is the chief cell of new tubercle formation ; and finally, the total leucocyte counts by themselves indicate roughly the amount of diseased tissues with which the leucocytes have to cope. This conception of the reaction to tuberculosis explains the wide variation of blood picture which may be found.

In the present cases the eosinophils do not differ significantly from the controls but the other cell types do. The polymorphs are increased ; lymphocytes and monocytes are diminished. This is probably due to the incidence of secondary infection as the majority of the cases were well advanced, and only a few early ones were included. The mean polynuclear index was 1·34. COOKE and PONDER'S mean index for ninety tuberculous patients in Britain was 1·82. Their standard deviation is not given, but on calculating it from their distribution it is found that the difference is significant. COOKE and PONDER'S tuberculous group was a miscellaneous one comparable to the Iraq cases. There is then, a greater " shift to the left " in tuberculosis in Iraq than in Britain, and so the deviation in the latter was also greater than was found by KENNEDY and FLINT (1930) in surgical tuberculosis in Switzerland, *a fortiori*, the Iraq results are still more " left-handed " than the Swiss.

Leprosy.

STITT (1929) says that other than a secondary anaemia, the blood picture is not characteristic, and BYAM and ARCHIBALD (1922) state it is of no particular significance. An as extensive as possible search through the large periodical literature on leprosy yielded only one paper on the white cells. ARIZUMI (1933)

states that the neutrophils are increased, the lymphocytes decreased, and the eosinophils few but may be increased. The cases in Iraq showed the neutrophils diminished, the polynuclear index slightly but significantly left-handed. Eosinophils and lymphocytes were increased, the latter giving the highest mean of all groups, the monocytes were not significantly altered. One cannot exclude the possibility that the eosinophilia may be in part due to coincident helminthic infection. Though the stools of all the cases were examined for ova, and positive ones omitted from the group, I did not conduct this examination personally. Although only about half the cases showed any basophils, the average per case was 0·775 as against 0·067. Admittedly the numbers are very small and the statistical significance difficult to estimate, but one gains the impression that these cells are distinctly more common in lepers. The chief character of the leprous blood picture in Iraq is a lymphocytosis. Considering that the disease is of the granulomatous type, and that the deviation of the polynuclear index is small, one may conclude the lymphocytosis is at the expense of the neutrophils. A few total white counts did not show a significant leucocytosis, which confirms this view.

Phlebotomus Fever.

This is common in Baghdad and most European residents suffer an attack in their first year of residence. The leucocyte state is usually given as a leucopenia with absolute neutropenia.

In this series all the means differed significantly from the controls excepting the lymphocytes ; there was a neutropenia and monocytosis, while the eosinophils were reduced to less than half the normal figure. Had all the blood specimens been taken at the end of the first day the effect would probably have been greater. A number taken on the second and third days showed a tendency for the leucopenia and monocytosis to be less marked. A striking character of the white cells was the large proportion which showed degenerative changes. Smear and basket cells were common ; the granulation of many neutrophils was deficient and even absent, and numbers of monocytes were vacuolated or had pale, diffuse cytoplasm and lightly staining nuclei, and the chromatin network had a washed-out appearance. Equally striking was the exceedingly low polynuclear index. The deviation was the most left-handed of all the groups.

Although the fever is short and never fatal, the disease is characterized by an aftermath of depression both mental and physical which may last for months. This is more understandable when it is seen what a profound alteration in the blood picture results from an attack.

Bilharziasis.

Besides the general appearance of a secondary anaemia, the characteristic blood reaction of this disease is eosinophilia. In this series, the mean was 25·9 compared

with 3·6 in the control, and the range was 9·5 to 56·5 per cent. This wide range in the relatively small number of cases flattened the frequency histogram but with regrouping the data or multiplying the number of subjects an approximation to the normal curve would probably be obtained. The neutrophils were diminished and the index deviated significantly to the left but less so than any of the other groups. The smallness of the deviation is interesting in view of the severity of the condition in the majority of the cases. The lymphocytes were reduced slightly and the monocytes considerably. The absence of any marked leucocytosis leads one to the opinion that the eosinophilia is at the expense of the other cells. Over half the blood counts included basophils, and the percentage of basophils per case though absolutely low (0·46) was much higher than the control group value (0·067). Though no exact figures are available, one has the impression from these and other observations, that basophils tend to be more numerous in cases with eosinophilia.

Conclusions.

The aim of this communication will have been attained in the main if it succeeds in demonstrating the need for accurate measurement of haemic variability in a population before standards of normality can be established. Extreme ranges and arithmetic means do not present an adequate picture of variability, and it is only by the application of statistical methods that a proper representation may be got. Data derived in this way are particularly necessary if different populations are to be compared whether they are of varying kind in the same locality, or of the same kind in different localities.

1. The differential leucocyte count and polynuclear index of 400 carefully controlled healthy persons in Iraq (the majority being British airmen) are described and statistical tests have been applied to the data. The statistics show the variation of the population is consistent with the normal curve of frequency distribution, as would be expected on general physiological grounds.

2. On comparing the results with an estimate derived from standard text-books of the differential blood picture in temperate climates, it is found that in Iraq the percentage of neutrophils is decreased, the percentage of eosinophils, lymphocytes and monocytes is increased. The polynuclear index is significantly lowered or deviated to the left.

3. The range of normal variation is much wider than is given in any of the available text-books.

4. The incidence of so-called abnormal cells, or cells not usually present in normal blood, is found to be greater than reported in standard works. This is taken to be evidence of irritability or greater activity of the haemopoietic system, and the contention is supported by the deviation of the polynuclear index.

5. It is suggested that the cause of the alterations in the blood are climatic but the detailed discussion of the evidence for this is left to a future paper.

No other generally acting factor could be found, to which the blood changes could be ascribed, though the possibility of such a factor existing is recognized.

6. Similar studies giving statistics and comparative standards are presented on the blood in malaria, tuberculosis, leprosy, phlebotomus fever, and bilharziasis. As would be expected in all these diseases the blood picture differs significantly from the control group. The polynuclear index shows a shift to the left as compared with the normals, and it is concluded that the value of this test is not invalidated by the deviation of the control group from British standards for under pathological stimuli it becomes *a fortiori* greater.

7. It is concluded that in Iraq at least, monocytosis is not a diagnostic characteristic of malaria.

8. Considerable variation is found in the differential count of the tubercular cases, and the impression is gained that the ranges are greater than those in similar cases in Britain. The polynuclear count is also significantly deviated to the left as compared with a British group of tubercular patients.

9. Relative lymphocytosis is a characteristic of the blood of lepers in Iraq. It is remarked that basophils are commoner in lepers than in the other groups.

10. The leucocytic alterations in phlebotomus fever are profound and attention is drawn particularly to the very low polynuclear index, and to the prolonged after-effects of the disease.

11. The wide range of eosinophils in bilharziasis is noted. The polynuclear count is just significantly deviated.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

THE TREATMENT OF SLEEPING SICKNESS WITH NEOCRYL.*

BY

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At the request of the Therapeutic Trials Committee of the Medical Research Council, the " Crylarsan " brand of neocryl was tested clinically in a number of cases of sleeping sickness in the region of Bolobo.

Neocryl (sodium succinanilomethylamide-*p*-arsonate) was originally prepared for the Chemotherapy Committee of the Council by Sir GILBERT MORGAN and his colleagues at the Chemical Laboratory, Teddington (Department of Scientific and Industrial Research). It was first tested by WARRINGTON YORKE, MURGATROYD *et al.* (1936),† who reported favourably on its actions in experimental trypanosome infections in animals, in human syphilis and in cases of Nigerian sleeping sickness. The " Crylarsan " brand of the compound has been supplied to us in generous quantity by Messrs. May and Baker, Ltd., for the purposes of the trials described below.

Selection of Cases.

As far as possible consecutive cases were taken, the only condition being that there should be a fair possibility of following up the case for a number of years, so that later it might be possible to give a report on the ultimate results of treatment with neocryl.

Classification and Control of Cases.

The series includes both first and second stage cases, while, in addition, there are three cases previously treated and found to be unresponsive to tryponarsyl (the Belgian equivalent of tryparsamide). Diagnosis was made in most cases by gland puncture, but in some cases the diagnosis was made on the symptoms and a suggestive cerebrospinal fluid, i.e., a high cell count of lymphocytes, and in some cases vacuolated cells. Unfortunately, it was not possible to centrifuge the blood of these latter cases.

Lumbar puncture was performed at the time of diagnosis and again at the end of the course of injections. In a few cases the albumin content was estimated but in general the progress of the case was estimated by the change of the cell content of the cerebrospinal fluid. If at the end of treatment the cell-count

*A report to the Therapeutic Trials Committee of the Medical Research Council on twenty-four cases treated at the Baptist Mission Hospital, Bolobo, Belgian Congo.

†YORKE, W., MURGATROYD, F. *et al.* (1936). A new arsenical for the treatment of syphilis and trypanosomiasis. *Brit. med. J.* 1, 1042.

Treatment of Sleeping Sickness with Neocryl.

Summary of Results.

Group.	Case No.	Reg. No.	Sex Age.	Weight kg.	Examination at Diagnosis.		Treatment with Neocryl.			Examination at End of Treatment.		Observations on Result of Treatment.
					Gland Puncture.	C.S.F. Cells.	Dose in Grammes.	Grammes per kg. Body Weight.	Total Neocryl in Grammes.	Gland Puncture.	C.F.S. Cells.	
A	1	278/36	♀ 22	49	+	5	2·0 weekly	0·04	24	—	3	Condition good: no symptoms
First stage.	2	280/36	♀ 17	56	+	2	2·5 ,,	0·044	30	—	2	do.
	3	275/36	♂ 14	41	+	Blood	2·0 ,,	0·049	24	—	6	do.
	4	276/36	♂ 7	27	+	7	1·5 ,,	0·055	18	—	2	do.
	5	2/37	♂ 25	59	+	2	3·0 ,,	0·05	36	—	5	do.
	6	254/36	♂ 25	53	+	15	2·5 ,,	0·048	30	—	2	do.
B	1	282/36	♂ 16	59	+	18	1·5 then 2 twice weekly	0·03 twice weekly	27·50	—	5	do.
First stage.	2	286/36	♀ 28	61	+	23	2·0 twice weekly	0·03 twice weekly	32	—	5	do.
	3	299/36	♂ 22	61	+	10	2·0 twice weekly	0·03 twice weekly	30	—	Blood	do.
C	1	262/36	♂ 22	63	—	420	3·5 weekly	0·055	42	—	30	Feels better: reported 6 weeks after treatment not feeling well, C.S.F. = 12
	2	251/36	♀ 10	225	—	330	1·5 ,,	0·07	18	—	130	6 weeks after treatment, onset of fits: sleeping: C.S.F., approx. 750 cells and trypanosomes present—relapse

Second stage.	3	264/36	♂ 20	55	—	950	3·0 „	0·055	36	—	16	No longer complains of symptoms
	4	259/36	♀ 45	56	—	253	3·0 „	0·05	36	—	30	Feels well but control lumbar puncture 6 weeks after treatment = 120 cells
	5	287/36	♀ 12	275	+	34	2·0 „	0·07	24	—	4	Feels well: no symptoms
	6	285/36	♀ 32	50	+	258	2·0 twice weekly then 3·0 weekly	0·06	33	+	32	Feels well: treated with 2 × 1·0 grammes Bayer 205 to sterilize blood
	7	300/36	♂ 32	61·5	—	247	3·5 weekly	0·055	42	—	18	Feels well: no sleeping
	8	304/36	♂ 30	63	+	55 & Tryps.	9 × 3·5 „ 3 × 3·0 „	0·055	40·5	—	12	Feels and seems well
	9	306/36	♀ 30	41	—	316	2·5 „	0·06	30	—	38	Feels better: still some headache
	10	305/36	♂ 14	34	+	200	2·0 „	0·06	24	—	58	Feels and seems much improved
D Second stage.	1	283/36	♂ 30	70	+	43	3·0 twice weekly	0·04 twice weekly	39	—	44	Feels well
	2	8/37	♀ 30	47	+	150	2 × 3 weekly then 2 twice weekly	0·04 twice weekly	30	—	24	No sleeping: general condition improved
E Relapsed cases.	1	281/36	♂ 25	62	—	72	4·0 weekly	0·06	20	Refused		After 20 grammes ocular trouble: general health and vision deteriorating
	2	279/36	♂ 30	53	—	80	3·5 „	0·07	35	—	205	Bad general condition after treatment: control lumbar puncture 1 month, after treatment = 220 cells
	3	245/36	♂ 12	35	—	42	1·5 twice weekly	0·04 twice weekly	75	Too ill		Course discontinued after 7·5 grammes. General condition deteriorating

in the cerebrospinal fluid was nearly normal, i.e., between 5 and 10 cells per c. mm., then rest from treatment was given for 6 months; if, however, the count was definitely abnormal, a rest of 1 to 3 months only was given.

Treatment and Dosage.

The dosage of the drug was calculated on the body weight of the patient, corresponding closely with the method described by CHESTERMAN (1931) for tryparsamide.

Dosage in grammes per kg. body weight.		
1*st Stage*:	Child	0·07
	Young person ...	0·055
	Adult	0·045
2*nd Stage*:	Child	0·09
	Young person ...	0·07
	Adult	0·06

The maximum dose used was 4·0 grammes and the maximum total dosage used was 42 grammes. Normally a series of 12 weekly injections were given, but in five cases (three first stage and two second stage) injections were given twice weekly and then it was calculated that the total weekly dosage should exceed the calculated dosage by 1 gramme, e.g., if the calculated dose by body weight was 3 grammes, then 2 gramme doses were given twice weekly, i.e., a total of 4 grammes in the week. In one case 3 gramme doses were given twice weekly.

A 40 per cent. solution of neocryl was used intravenously.

It may be noted that normally the form of sleeping sickness with which we deal reacts very well to arsenic in the form of tryponarsyl.

Clinical Records of Cases under Observation.

For the purposes of description, the cases are divided into five groups, and a summary of the results of treatment is given in tabular form on pages 334 and 335.

Group A.—First stage, with weekly injections. Cases with a cell count in the cerebrospinal fluid of up to fifteen cells were considered as in the first stage.

Group B.—First stage, with injections twice weekly. In this series one early second stage case (B2 in the series) was treated as a first stage case.

Group C.—Second stage, with weekly injections.

Group D.—Second stage, with injections twice weekly.

Group E.—Cases previously unresponsive to arsenical treatment.

Reactions and Toxic Effects.

In some of the cases, Cases A3, A4, C2, the temperature was taken 3 hours after the first injection; in no case was it raised by more than 1° C. upon the resting temperature.

In none of the cases recorded in Groups A, B, C, D, was any toxic symptom recorded, except in Case C6, in which there was an indefinite history of dimness of vision. In one case, Case D2, a dose of 3 grammes of neocryl was given twice weekly without untoward effect.

In Group E, cases previously treated with arsenical compounds, there was one definite case (Case E1) of severe ocular symptoms.

Discussion of Clinical Results.

It will not be possible to give any definite estimate of the value of neocryl until the results of a single course of treatment have been followed up for a period of years. From the immediate results obtained in this series, it is possible to form a rough comparison of neocryl and tryponarsyl, since dosage was similar to that used in routine treatment with the latter drug.

In first stage cases the immediate results are satisfactory clinically and from the laboratory point of view: they compare favourably with those with tryponarsyl.

In second stage cases the immediate results are not so satisfactory, and do not compare so favourably with those treated with tryponarsyl. It is usual, with the dosage of tryponarsyl that we employ, that the cell count in the cerebro-spinal fluid is reduced very considerably (usually below twenty cells per c. mm.) at the end of a single course of 12 weekly injections. It will be noted that this occurs in a number of the recorded cases with neocryl, but the reduction is not so marked, nor does it occur regularly. After treatment, with tryponarsyl rapid relapse is, in our experience, not common, and a single course if it does not give a complete cure, will usually give a year's freedom from relapse. In two of the cases recorded, Cases C2 and C4, there are signs of relapse occurring 2 months after treatment with neocryl.

In one case (Case C6) trypanosomes were persistent in the gland juice after 33 grammes of neocryl had been given. This persistence has, however, been noted rarely with tryponarsyl, say, once in every 300 cases.

Although perhaps the number of cases treated with injections twice weekly is not sufficiently large to give a fair comparison, yet no advantage was noted with the more frequent treatment, rather the contrary (*vide infra*).

Throughout the series one has had the impression that neocryl is a less toxic drug than tryponarsyl, and in view of the large dose given with impunity in Case D1 (3 grammes twice weekly) it is possible that larger doses than those used in tryponarsyl treatment might be given with safety, and perhaps with advantage.

Summary.

1. A series of twenty-one cases of sleeping sickness were treated with neocryl with dosage similar to that of tryponarsyl, the Belgian equivalent of tryparsamide: three cases previously unresponsive to arsenical compounds were similarly treated.

2. In first stage cases there was definite clinical improvement, and the immediate results as determined by gland and lumbar puncture were satisfactory.

3. In second stage cases despite marked clinical improvement, the immediate results were not so satisfactory. The result of the treatment in these cases seems uncertain, in view of the rapid relapse of Cases C2 and C4, and the trypanosomes persistent in the blood of Case C6. In reference to this last case it is worthy of note that in the blood of some cases treated with tryponarsyl in our district trypanosomes can be found even after a full course of the drug.

4. In cases previously treated by arsenical compounds the results were not encouraging.

5. No advantage was found in giving injections twice weekly, although the number of cases treated thus does not perhaps permit a fair comparison.

6. No definite toxic reaction was noted, except in cases previously treated with tryponarsyl. A dose of 3 grammes twice weekly was given without untoward effect in a normally developed man.

7. It is impossible to compare the result of treatment with neocryl with that of tryponarsyl (tryparsamide) until the cases reported have been followed up for at least 2 years, but the evidence submitted in the addendum suggests that it is definitely inferior to tryponarsyl.

In conclusion I wish to thank M. LE MÉDECIN EN CHEF, of the Medical Service, Belgian Congo, for permission to test neocryl on the patients under my charge at Bolobo.

ADDENDUM.

Since the above paper was sent for publication, it became evident that there were many relapses. Accordingly I re-examined all the test cases before 6 months had elapsed after their courses of treatment. The following gives a brief idea of the results :—

Of nine first-stage cases, two have had to be taken under treatment again because of raised cell count in the cerebrospinal fluid.

Of twelve second-stage cases, either seven or eight have relapsed and have been taken under treatment again.

It is interesting to note that the two first-stage cases which relapsed were those that were treated by two injections weekly. This is a remarkably high relapse rate, and the symptoms have in most cases returned. It is usual for a patient to say that he had relief from the symptoms for perhaps a month, but then they returned. On the whole neocryl is apparently a somewhat dangerous drug to use in sleeping sickness, as it gives rather rapid clinical improvement which, however, is only temporary in second-stage cases; the danger is in the treatment of patients who cannot be adequately followed up, and who, as in the case of some of those reported by LESTER in the paper by YORKE, MURGATROYD, *et al.*, disappear before the end of treatment.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

ENDEMIC ENLARGEMENT OF THE PAROTID GLAND IN EGYPT.

BY

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INTRODUCTION.

It is now 49 years since MIKULICZ (1888) demonstrated the first case of the disease now called after his name and since known to be a hyperplasia of the lymphadenoid tissue of the salivary and lachrymal glands. Recently certain other affections of the salivary glands, especially the parotids, have been described: a recurrent pyogenic parotitis by PYRAH and ALLISON (1931), PYRAH (1933) and PAYNE (1933), as well as a non-infective type of recurrent enlargement described by PEARSON (1935 and 1936). This last is associated with allergic conditions but may be complicated by secondary infection.

Endemic parotid enlargement in Egypt differs from the above-mentioned forms in its clinical and pathological features. It is a chronic painless hyperplasia common among agricultural workers, who are generally unaware of its presence.

Various medical men in Egypt have referred to this condition. SANDWITH (1905) attributed it to chronic parotitis or to some obstruction of Stenson's duct.

*I must record my grateful thanks to Prof. H. B. DAY for his constant help and criticism; to Prof. A. F. BERNARD SHAW for his encouragement and for the pathological studies, and to Prof. M. OMAR BEY for the help of his Department of Clinical Pathology in many of the investigations.

DAY (1933) described it as occasionally associated with endemic (bilharzial) cirrhosis, while BIGGAM and GHALIOUNGUI (1934) noticed it among *Ankylostoma* cases.

KIEVIET DE JONGE (1928) described " a curious broadness of the face recalling parotitis " in beriberi. He stated that the parotid glands are enlarged and the enlargement is not due to oedema. MILLER (1925), working on pellagra, noticed the condition and offered an explanation which is discussed later.

In Madagascar a parotid enlargement known there as " mangy" was described by FONTOYNOT (1911). Some cases are familial or hereditary and apparently resemble the Egyptian form. It is said to occur in localities resembling " goitre belts " and FONTOYNOT ascribes it to " a low grade regional infection."

CLINICAL FEATURES.

The following account is based on a study of a hundred cases admitted to hospital for various diseases.

Age.—Varies widely; the youngest was a boy of 10 years, the oldest a man of 65. Most patients were between 20 and 40 years of age.

Sex.—Males strikingly predominated. Only seven cases were in females.

Family.—Three patients only could tell of a family incidence.

Residence.—Most of the patients came from Lower Egypt, a considerable proportion from the neighbouring province of Giza.

Occupation.—All were agriculturists or field workers.

Habits.—None of chewing were present. Only a few patients who harboured ankylostomes were mud-eaters.

Diet.—This consisted mainly of maize bread, salted cheese and onions; possibly its salt content might initiate much salivation.

Onset.—No patient gave a history of acute parotitis; the onset of the parotid swelling was gradual in the minority who were aware of the condition; and in one case was watched in the early stage.

Symptoms.—The condition gave rise to no complaints from the patients, who were admitted for some associated disease. Only five had noticed sialorrhoea; two of these harboured ascarids which might be responsible.

Local Signs.—The swelling of the cheek corresponded with the anatomical boundaries of the parotid. In eighty-two cases both parotids were enlarged, generally equally, though in ten patients the glands were of unequal size. The enlargement varied in degree; when the swellings were marked it gave a triangular appearance to the face, hiding the lobules of the ears (Fig. 1, *a* & *b*). The consistency was usually soft but tended to be firm when the gland was much enlarged. In one diabetic patient the glands were nodular. In eighteen of the hundred patients the enlargement was unilateral; no less than sixteen of these cases were pellagrins.



FIG. 1*a*.



FIG. 1*b*.



FIG. 2*a*.



FIG. 2*b*.

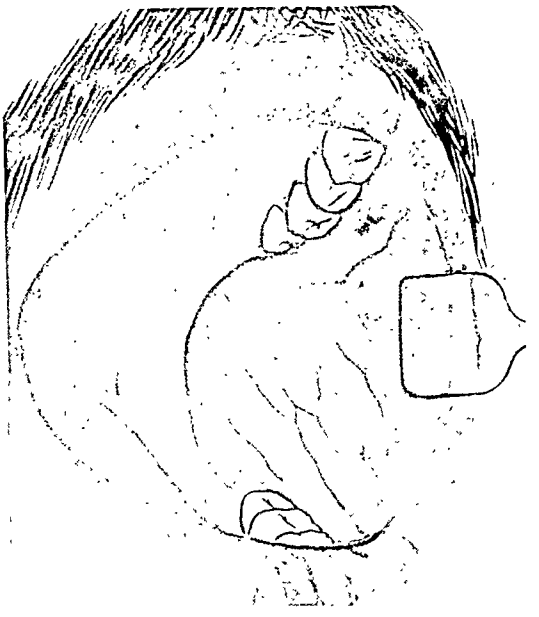
FIG. 1*a*.—Bilateral parotid enlargement, giving a triangular appearance of the face and hiding the lobules of the ears.

FIG. 1*b*.—Profile view of same. The left submaxillary gland is also enlarged.

FIG. 2*a*.—Bilateral parotid enlargement before X-ray treatment.

FIG. 2*b*.—Same, showing marked regression of enlargement after application of X-ray therapy.

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From drawing by W. Stiek.

FIG. 3.

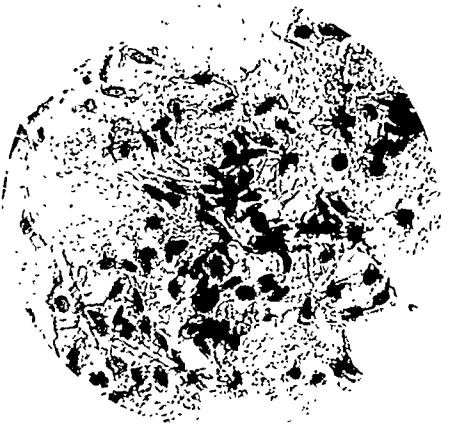


FIG. 4.

FIG. 3.—Mucous membrane, left cheek, showing fissuring.
The opening of Stenson's duct looks normal.

FIG. 4.—Flask shaped and squamous epithelial cells in catheterized saliva, from a case of parotid hyperplasia and pellagra.



FIG. 5



FIG. 6.

FIG. 5.—Sialogram showing marked dilatation of the main branches. Some tortuosity is seen.

FIG. 6.—Sialogram showing a "leafless tree" appearance and dilatation of the small ductules.



FIG. 7*a*.



FIG. 7*b*.



FIG. 8*a*.



FIG. 8*b*.

FIG. 7*a*.—(Low power.) Photomicrograph from a normal parotid.

FIG. 7*b*.—(Low power.) Photomicrograph from a case of parotid hyperplasia of same age as FIG. 7*a*, showing hyperplasia of the ductules and compactness of the acini.

FIG. 8*a*.—(High power.) Photomicrograph of normal parotid.

FIG. 8*b*.—(High power.) A mild degree of hyperplasia from another case of enlarged parotid.

Inspection of the mouth showed no constant change in the appearance of the mucosa nor in the opening of Stenson's duct. In some cases, generally pellagrous, there was fissuring or pigmentation inside the cheek; sometimes both (Fig. 3). Ten patients had pyorrhoea, but in general there was no relation between any mouth infection and parotid enlargement.

Other glands.—The submaxillary glands were enlarged on both sides in eight cases only. The lachrymal glands were enlarged in one case.

ASSOCIATED DISEASES.

There was no relation between the parotid condition and the general state of nutrition. The following table shows the associated diseases in one hundred cases :—

ASSOCIATED DISEASES IN 100 CASES.

	Number.		Number.
Pellagra	65	Pure *Ankylostoma* anaemia ...	4
Diabetes (manifest or latent) ...	10	Heart failure	4
Cirrhosis of liver	6	Various (1 case of each)... ...	11

This table shows that the commonest disease associated with endemic parotid enlargement is pellagra (65 per cent.); the next being diabetes (10 per cent.). Owing to the occurrence of parotid enlargement in diabetics in other countries, this group must be considered separately, for which purpose the various associated diseases in the above hundred cases may be grouped as follows :—(*a*) a pellagrous group, sixty-five cases; (*b*) a simple (non-pellagrous) group, twenty-five cases; (*c*) a diabetic group, ten cases.

The parotid enlargement was bilateral in all the diabetic cases, while sixteen of the eighteen cases of unilateral involvement occurred in pellagrins. In the latter group the manifestations of pellagra varied in severity; two patients also had cardiac beriberi.

Groups (a) and (b).

To determine the influence of other possible factors, an analysis of the cases in these groups gave the following incidence of various infestations :—

	(*a*) Pellagrous group. 65 cases.	(*b*) Simple non-pellagrous group. 25 cases.
Cirrhosis and splenomegaly	25	6
Intestinal parasites	42	8
Ankylostoma	24	3
Schistosoma mansoni	18	5
Ascaris ...	13	0
Hymenolepis nana	0	1

From the figures it is evident that pellagra is the commonest disease associated with the condition, and that cirrhosis and ankylostomiasis are present in less than half the total number.

Anaemia.—There was no definite relationship between parotid enlargement and the degree of anaemia. Haemoglobin values varied from 12 to 90 per cent., and the red cell count from 1,250,000 to 5,050,000. The white count varied from 3,000 to 9,000.

Gastric Function.—The gastric function was investigated in forty-five cases; high acidity was found in five; absence of free acid in eight; hypochlorhydria in five; low normal curves in seven and normal curves in twenty cases. This variation seems to exclude any relationship between parotid enlargement and derangement of gastric functions.

The Diabetic Group.

Five cases had manifest diabetes; one of these was insulin-resistant. Five other cases are included in this group; they had diminished sugar tolerance but no glycosuria nor obesity.

FLAUM (1932) described a condition of diminished sugar tolerance without glycosuria, associated with parotid enlargement; his cases occurred in middle-aged, hypertensive and obese persons. To separate endemic parotid enlargement from FLAUM'S type in latent diabetes, it was found essential to test the sugar tolerance in a series of cases. Owing to some well-known abnormalities met with in the oral glucose tolerance test when used in cases of pellagra, dysentery and ankylostomiasis, the intravenous glucose tolerance test was employed to check the results. It was found that endemic parotid enlargement is not associated with any disturbance in the sugar regulating mechanism, with the exception of the ten cases described as the diabetic group. Further, the constitutional type of the patients differed from those in FLAUM'S series.

Relation to Pancreatic Function.

In selected cases the efficiency of pancreatic digestion was tested by the method of " nuclear digestion " using Schmidt's diet or thymus gland. Of ten cases, only two showed diminished pancreatic efficiency; eight showed good digestion.

Diastatic Index.—DUNLOP (1933) recorded a rise in the diastatic index in sixty cases of mumps. The diastatic index was estimated in a series of twenty-eight cases of endemic parotid enlargement. Only eleven cases gave readings above 30; two of them gave 32, the others between 40 and 112 units. Three of the high readings were in cases complicated by bilharzial cirrhosis, and can be excluded because of the possibility of bilharzial disease of the pancreas (DAY, 1933). Therefore only six cases of pure parotid enlargement showed a definite rise in the diastatic index out of the twenty-eight

examined. Whether this rise indicates a pancreatic affection or not is uncertain ; no involvement was found in cases examined at autopsy, and the complication, should it occur, appears to be quite unusual. Renal disease was excluded in cases showing a normal index.

INCIDENCE.

The incidence of parotid enlargement was investigated at two provincial hospitals devoted to the treatment of endemic diseases.

	Total.	Male.	Female.
Shanshour Hospital—			
Number of patients seen	4,076	2,157	1,919
,, of parotid cases	80 (2%)	69 (3·5%)	11 (0·5%)
Kalleen Hospital—			
Number of patients seen	1,585	1,041	544
,, of parotid cases	24 (1·5%)	23 (2·2%)	1 (0·2%)

It should be mentioned that at Kalleen ankylostomiasis is rare, but parotid enlargement common.

Incidence among Pellagrins.—This was determined approximately from the admissions to Kasr-el-Aini Hospital from November, 1935, to October, 1936. Of 201 cases of pellagra admitted, sixty-five had parotid enlargement, giving an incidence of 32·2 per cent.

STUDY OF THE SALIVA.

Cytological and Bacteriological.—Microscopic examination of the catheterised saliva from Stenson's duct occasionally showed peculiar flask-shaped cells, a few pus cells and squamous flat cells in cases of parotid enlargement, especially when pellagra was present. Some cases of severe pellagra without parotid enlargement gave similar findings. The saliva was always clear and alkaline in reaction. No organisms were seen and cultures were uniformly sterile. The microscopical picture is shown in Fig. 4.

In the recurrent pyogenic parotid affection described by PAYNE these flask-shaped cells were also seen, and sometimes " duct casts " ; but organisms were also demonstrated.

Physiological.—The following investigations were made :—

A—Estimation of the ptyalin content of the saliva in cases of enlarged parotids of varying degree, with different associated conditions, and also in a series of normal controls. B—Determination of the response of enlarged and of

normal parotids to pilocarpine. C—Estimation of the total solids and of the calcium content in normal and enlarged parotids. D—Estimation of the thiocyanate content of the saliva from normal and enlarged parotids.

A—Ptyalin Content of Parotid Saliva.—COLE'S method (COLE, 1926) was used. The range of the ptyalin content in thirty-one normal Egyptians varied between 4,000 and 31,000 units per 100 c.c. saliva. One dark coloured individual gave a higher range (72,000 units), and some diurnal variations were also observed. In twenty-nine patients with parotid enlargement (including diabetics) the range varied from 4,400 to 40,000 units. Two cases of mumps gave figures of 11,200 and 16,000 respectively.

There is therefore no diminution of the ptyalin content of the parotid saliva of patients with parotid enlargement as compared with normal Egyptians.

B—Response to Pilocarpine.—Saliva was collected by catheter after injection of pilocarpine and drainage maintained for half an hour. The average response in normal controls was 24·7 c.c. per 30 minutes, and that of enlarged parotids (diabetics included) 42 c.c. This shows that the secretion of saliva in cases with enlarged parotids is far greater than that of the normal gland. The output was nearly proportionate to the degree of enlargement. It is interesting to find that very few of these patients complained of sialorrhoea.

C—Total Solids and Calcium Content.—The average total solids in saliva from enlarged parotids was 0·57 gramme per cent. and the calcium 6·9 mg. per cent.; from normal controls 0·65 gramme and 7·9 mg. respectively. This shows lower figures for enlarged parotids. The difference, though small, could be accounted for by the invariable absence of potassium thiocyanate in cases of enlarged parotids (*vide infra*), and also possibly by the normal variability of the calcium content of saliva.

D—The Thiocyanate Content.—This was estimated in a series of ten patients only of the endemic group. The normal range in controls varied between 0·00025 and 0·005 per cent., while in cases with enlarged parotids it was totally absent from parotid as well as from mixed saliva. This was proved to be due to deficiency of thiocyanate and not to failure of its excretion by the salivary glands, for when thiocyanate or thiosulphate was administered, thiocyanate was demonstrated in saliva from enlarged parotids in amounts within the normal range.

RADIOLOGICAL STUDY.

Sialography with lipiodol injected into Stenson's duct by a Luer needle and record syringe was performed on a series of patients, and also on normal controls. The following appearances were found and are illustrated :—

(*a*) Spherical dilatations of the ends of the small ducts. This " bronchiectatic " appearance was seen also by PAYNE in recurrent pyogenic parotitis and by PEARSON in the allergic type.

(*b*) Fusiform and segmental dilatations affecting the medium and larger

ducts occurred in 8 per cent. of cases; some tortuosity of the duct was also noticed (Fig. 5).

(*c*) Another picture presented may be termed the "leafless tree" type. The medium ducts and ductules were so numerous as to suggest fresh formations. In some cases the main duct was varicose (Fig. 6).

(*d*) In two cases only of parotid enlargement (one in a diabetic, the other in a pellagrin) there was a filling defect.

(*e*) Fifty per cent. of cases gave appearances which differed little from the normal sialogram, except in demonstrating the large size of the gland. In some the acini were quite conspicuous, suggestive of dilatation.

MORBID ANATOMY.

Material was obtained from three postmortem cases and two biopsies. In the former the weight of the parotid was two to four times that found in normal control specimens. No naked eye evidence of cysts or neoplasm was found.

Histological examination showed a striking increase in the number of ducts and ductules, some of which were ectatic. The secretory part of the gland looked more compact and the acini more crowded. (See microphotographs Figs. 7 (*a*, *b*), 8 (*a*, *b*). There was no proliferation of the duct epithelium, in contrast to that found by PAYNE in recurrent parotitis. No signs of inclusion bodies, neoplastic growth nor scarring was seen. The appearance was that of a hyperplasia of an actively functioning gland. The degree of hyperplasia varied in different cases; in two biopsy specimens, removed from the superficial portion of the parotid, the histology was almost normal. This coincides with the normal type of sialogram obtained in 50 per cent. of the cases.

Examination of the pancreas in the autopsy cases showed neither naked eye nor histological abnormality.

For the sake of analogy, hypertrophied sebaceous glands from pellagrins were compared with normal controls. No differences in histological structure were found.

On the evidence collected it was justifiable to call the endemic parotid enlargement a "parotid hyperplasia."

ETIOLOGICAL CONSIDERATIONS.

These investigations gave some indications of the possible origin as well as the nature of the disease. It appeared that endemic parotid enlargement was not of inflammatory nature and that the hyperplasia could not be attributed to ankylostomiasis or other infestation. Clinical evidence connected the condition with pellagra and with diabetes.

Pellagra.—This disease might act as an etiological factor in various ways:—

(*a*) By representing or producing some deficiency state to which the

parotid glands respond by undergoing hyperplasia, analogous to enlargement of the thyroid in iodine deficiency.

Parotid enlargement has been described by DE JONGE (1928) in beriberi; which suggests that the vitamin B_1 complex might be the deficient factor, in the absence of which the parotids undergo hyperplasia of a compensatory character. Some doubt was thrown on this hypothesis because parotid enlargement developed in one of the Egyptian cases during treatment with marmite.

(*b*) Parotid enlargement might be related to the absence of potassium thiocyanate from the saliva. According to A. CLARK (personal communication) this sulphur compound is excreted in excess in the early stages of pellagra and so might induce an overwork hypertrophy. The bactericidal action claimed for thiocyanate might predispose, in its absence at a later stage, to infection by some mild agent. At present the significance of the variations in salivary thiocyanate which are found in pellagra is uncertain.

(*c*) Experimentally a parotid hyperplasia in guineapigs was obtained by WARTIN and FLORENTINE (1927) on exposure to X-rays. The exact mechanism is unknown. It is doubtful if this observation has any bearing on pellagra.

(*d*) The local changes in pellagra affecting the buccal mucosa, the papillae and main duct (desquamation), combined with a lowering of the body resistance from malnutrition, might pave the way for some unknown infective agent.

Diabetes.—This could also be regarded as a predisposing factor. Parotid enlargement only occurs in a small percentage of diabetic patients and is unrelated to the severity of the disease. FLAUM regards parotid enlargement as a symptom of diabetes, manifest or latent, in obese middle-aged persons. He considers that the parotids have an internal secretion which has the property of increasing sugar tolerance. In one diabetic, referred to the writer by Prof. ISMAIL PASHA, the parotid enlargement was associated with infected teeth which were all removed; therefore the possibility of infection in diabetes should be considered.

Other Factors.—ULTIMURA (1927), following German writers, believes that the salivary glands are organs of internal secretion. A deficiency state, which may often be associated with pellagra, might be responsible for parotid hyperplasia. This theory is purely hypothetical and there is no clinical connection of parotid enlargement with endocrine disease.

That a salty diet might cause excessive salivation and lead to parotid hyperplasia did not appear a likely explanation. This diet is common to a large section of the population and the patients made no complaint of salivation.

The possibility that an irritant or toxic agent is excreted in the saliva and provokes an enlargement is difficult to accept. Such agents would tend rather to depress the functions of the gland and lead to degeneration, not to hyperplasia.

Alternative Theories.—Limiting consideration to the endemic group, there were two main theories to account for the parotid enlargement (1) compensatory hyperplasia, (2) some form of infection.

THEORY OF INFECTION ; TESTS.

The parotid gland is naturally liable to infection ; according to SEIFERT (1926) the secretion is lacking in bactericidal action owing to the poor content of mucin.

The infection theory would explain the unilateral enlargement found in one-fifth of the cases, and its occurrence in patients not suffering from any obvious deficiency. Malnutrition and the insanitary conditions under which the poorer classes live would predispose to infection.

The absence of signs of inflammation and the negative results of cultures excluded an ordinary bacterial infection, but there remained the possibility of some virus disease. According to KUTTNER and WANG (1934) there is a liability to virus infection of the salivary glands of guineapigs, hamsters and human beings. These viruses may remain dormant without causing any symptoms. While the effect of many virus infections is ultimate necrosis of affected cells, certain viruses cause a proliferation of parasitized cells producing hyperplasia or even neoplasia, as in the infectious papillomatosis of wild cotton-tail rabbits (SHOPE, 1933).

The source of such a possible infection was looked for in localities from which the patients came. The writer examined a large number of domestic animals from various parts of Egypt, but found no instance of parotid enlargement among them.

Allergic Tests.

Intradermal tests with saliva and parotid emulsion from affected patients were made on other cases and on normal individuals. The results showed no constant difference. The common association with pellagra diminished the value of these tests because pellagrins do not possess normal skin reactivity.

Animal Inoculation.

(1) Rabbits. Intracerebral inoculation of rabbits with catheter specimens of saliva from affected and control persons was performed, after Gordon's technique. Both classes of saliva occasionally induced a lymphocytic encephalitis or a meningo-encephalitis, apparently due to a contaminating virus.

To avoid contamination that might be present in saliva, the experiments were repeated with ground parotid gland tissue obtained by biopsy. No single rabbit developed encephalitis ; when the animals were eventually killed there was neither naked eye nor microscopical evidence of any abnormality.

(2) Guineapigs. At the same time injections of saliva and parotid emulsion from human cases were made into the parotid and submaxillary glands of guineapigs, on one side ; the other was left as a control. No sign of disease appeared, and on subsequent histological examination no difference in structure could be detected between the injected and control salivary glands.

(3) Monkeys—*Macacus rhesus*. To imitate the probable route of an

infection, a considerable amount of saliva from human cases (taken by catheter) was injected through Stenson's duct to the parotids of two monkeys. The procedure was similar to that of JOHNSON and GOODPASTURE in producing experimental mumps in *M. rhesus*. The two monkeys injected were kept for periods of 5 and 8 months respectively without result, and even intraglandular injection with human parotid emulsion failed to produce signs of disease.

The results of these experiments gave no support to the theory of infection as responsible for endemic parotid hyperplasia.

TREATMENT.

To supply any dietary deficiency, trial was made of various remedies as used for pellagra. A due supply of animal protein vitamins B_1 and B_2, sometimes supplemented with vitamins C and D was given to patients for varying periods without effect on the parotid enlargement. Iron and hydrochloric acid, with or without pepsin, were also useless. In view of the anatomical condition of the gland no rapid results could be expected from such measures.

Spontaneous regression occurred in one case, and also followed biopsy in two other patients. The possibility of the pressure of a bandage causing this regression was excluded. The enlargement subsided in another patient following an attack of erysipelas of the buttock. An analogy may be drawn between these examples and similar experiences in Mikulicz's disease. In cases of the latter affection a regression has been reported after erysipelas and other infections such as pneumonia and influenza ; also after removal of the lachrymal gland.

X-Ray Therapy.—Conflicting opinions on the value of this form of treatment in Mikulicz's disease have been expressed. But in view of the simple hyperplasia found in this endemic form, it was decided to apply the treatment, and the results were very satisfactory. Small doses at weekly intervals were applied, first to one side only, leaving the other side as a control. After regression of the swelling on one side, the other parotid was similarly treated. The course consisted of five doses. Out of ten cases treated, nine responded with regression in the size of the glands. There was no evidence of fibrosis nor disturbance of function, producing dryness of the mouth, after treatment. The result is illustrated in Fig. 2 (*a* & *b*).

DISCUSSION.

In view of the failure of all attempts to transmit the disease or to show the presence of an infective agent, it is extremely doubtful whether endemic parotid enlargement is of infective origin. No form of infection is known which produces uniform hyperplasia with full retention of functional efficiency in any organ. In spite of unilateral involvement sometimes found, the probabilities are strongly against an infective origin.

It is a familiar fact that most deficiency diseases with an endemic distribution or with sporadic outbreaks have been at some time attributed to an infection.

The reports of chronic parotid enlargement in widely separated countries associated with conditions of malnutrition, indicate that the hyperplasia occurs in response to some increased functional demand on the glands. This demand arises from some need of the body secondary to some deficiency.

Parotid hyperplasia does not appear to be related to any known alimentary disorder, such as gastric achylia or pancreatic disease. MILLER (1925) considers that an increased secretion of ptyalin is called for in pellagra on account of a diminution in amylopsin and pancreatic secretion generally. His view is that pellagra is characterised by achlorhydria which prevents the formation and action of Starling's secretin and so leads to pancreatic deficiency. This hypothesis fails to explain the frequent association of parotid hyperplasia with normal gastric function and its absence after complete gastrectomy.

Whether a primary dietary deficiency, either of B vitamins or of some other factor, is directly responsible is uncertain. The parotid enlargement did not usually regress in pellagrins when the general condition improved on special feeding, and it was found in some individuals who showed no malnutrition. The absence of thiocyanate may be etiologically significant and this subject requires further investigation.

The possibility that the parotids may furnish an internal secretion, as suggested by FLAUM and others, has been mentioned. There may even be some unknown constituent of saliva itself, analogous to Castle's intrinsic factor in gastric juice, that may play an important part in the body economy. Recently SYDENSTRICKER *et al.* (1936) have found that the administration of normal gastric juice causes marked improvement in pellagrins, even when kept on a pellagra-producing diet. SALAH (1935) has demonstrated the presence of Castle's intrinsic factor in the gastric juice of pellagrins ; therefore some other intrinsic factor must be postulated as absent in pellagra. It is possible that this is really derived from saliva and not from pure gastric secretion.

These suggestions must be left for future research to verify or reject.

SUMMARY.

1. A chronic painless enlargement of the parotids is common in Egypt ; in some districts about 2 per cent. of the poorer agricultural workers are affected.

2. Males are particularly affected ; about ten times as frequently as females. The usual age of the patients is between 20 and 40.

2. Of one hundred cases seen among hospital in-patients, sixty-five were associated with pellagra and with twenty-five others represented an endemic group. The condition was not specially associated with any other endemic disease ; in one-fifth of these cases the parotid enlargement was unilateral.

4. The remaining ten cases had manifest or latent diabetes ; this association has been noticed in northern latitudes.

5. In the endemic group the condition bore no relation to any derangement

of the gastric or pancreatic functions, or to the blood count. There was no disturbance of the blood sugar regulating mechanism in these cases.

6. The saliva occasionally showed abnormal cell elements and was sterile. Functional tests showed increased secretion with a full ptyalin content, but there was a striking absence of thiocyanate.

7. Sialograms were normal in half the cases. Abnormalities seen were dilatations of the main duct or ductules, or a multiplicity of ducts giving a " leafless tree " appearance.

8. Microscopical examination of the glands showed chronic hyperplasia with entire absence of any inflammatory changes.

9. Allergic tests and experiments on animals, including monkeys, failed to demonstrate an infective origin. Intracerebral inoculation of saliva was found unreliable because of the presence of contaminating viruses in normal and abnormal cases.

10. A satisfactory method of treatment by X-rays was applied.

11. The etiology is discussed and various theories reviewed.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

SPRUE IN EGYPT.

REPORT OF A CASE.

BY

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Since the description of the features of the disease by MANSON (1880) and VAN DER BURG (1880) and the adoption of its popular designation in Java as the medical name, the individuality of sprue has been established and its nature has been described by many authors.

There are two peculiarities about sprue, namely its patchy distribution between 40° north and 20° south, with the exception of Central Africa; and its occurrence mainly in Europeans sojourning in those countries. With the exception of the natives of Puerto Rico it was supposed to be limited to Europeans but recently it has also been reported in natives in India (1930, 1931). As the aetiology of sprue is not yet known, it is difficult to explain this peculiar distribution and racial preference. Until now no case has ever been recorded from Egypt.

The first case of sprue occurring in an Egyptian was detected in the Research Institute, Cairo, and is described below.

CASE REPORT.

Z.A.T., male, aged 40, was sent from the State Railways Hospital to the Research Institute on 16th February, 1936, for investigation.

Complaint.—Rapid wasting and general weakness, inability to take food because of pain in the tongue; diarrhoea, three to five semi-fluid, offensive stools daily, having the same colour as the food taken. Gurgling noises in the abdomen 2 hours after a meal.

History.—On 7th July, 1935, patient was suffering from cough without expectoration but accompanied by fever.

Ten days later he developed diarrhoea with occasional blood and mucus.

In December, 1935, he first noticed a burning sensation in his tongue and diarrhoea with three to five bulky and light coloured stools daily, 2 hours after taking food.

The patient was admitted to the State Railways Hospital where he was given medicine and light diet; there was a slight subjective improvement.

Physical Examination.

Patient is greatly wasted, weighs 47·5 kg. Skin dry and inelastic. No evidence of previous pellagra, no abnormal pigmentations.

Tongue red, glazed and fissured.

Abdomen retracted on admission. Liver not felt below the costal margin, liver dullness diminished. Spleen not felt. No masses or glands in the abdomen. Heart weak sound, no murmurs.

Blood pressure 110/70.

Chest free. No glands, no oedema.

Nervous system free with the exception of weak knee and ankle reflexes.

INVESTIGATIONS.

Haematological.—Haemoglobin 55 per cent.; Reticulocytes 0·8 per cent.; Red Blood Corpuscles 3,110,000; Diameter index 8·03; Vol. index 0·9; Fragility, normal; White Blood Corpuscles total 3,600; Differential Eosinophils 1 per cent.; Neutrophils 58 per cent.; Lymphocytes 36 per cent.; Mononuclears 3 per cent.

Bone-marrow megaloblastic, hypoplastic. No parasites could be demonstrated in the blood.

Biochemical.—Icterus index 3 units; Van den Bergh, negative; Urea, 25; Calcium 9 mg. per cent.; Sugar tolerance curve 71/87/100/95/75; Cholesterol, 90, free 50, 88, ester 38.

Serological.—Wassermann and Kahn reactions negative. Sediment rate + +.

Bacteriological.—Widal negative; Stool culture negative for pathogenic micro-organisms and tubercle bacilli.

Gastro-intestinal.—Test meal : rapid emptying, HCl absent with alcohol meal, only present after histamine.

Sigmoidoscopy.—Diffusely congested mucous membrane with occasional flakes of mucus, no ulcers. Scraping from bowel, negative for amoebae and pathogenic micro-organisms (by culture).

Stools light coloured, bulky and offensive.

Fat :—Total 60 per cent, combined fatty acids 58·9 per cent., neutral fat 1·1 per cent.

Urine.—No albumin, sugar, urobilinogen or bile.

Radiograph of stomach, colon, lungs, mediastinum and bones did not show evidence of disease.

Diagnosis.

The case was admitted with the provisional diagnosis of steatorrhea, ? pancreatic disease, ? sprue.

The demonstration of marked glossitis, macrocytic anaemia and the chemistry of the fatty stools excluded pancreatic disease and the case was put under the category of steatorrhea due to deficient absorption of split fat.

Obstructed lacteals due to tuberculosis, Hodgkin's disease or cancer were then excluded by the absence of any clinical or radiological evidence and also by the subsequent course of the disease. The presence of marked glossitis as a prominent feature of the case, the absence of megalocolon as well as absence of marked hypocalcaemia and osteoporosis were rather in favour of tropical sprue than of idiopathic steatorrhea manifesting itself late in life, if the separation of the two conditions is to be admitted.

Pellagra is associated with diarrhoea, anaemia, glossitis and wasting and thus may be confused with sprue. The difficulty of their differentiation in endemic areas has been discussed (McLester, 1930). The absence of any history or evidence of cutaneous manifestations exclude this possibility in our case. Moreover, the anaemia of pellagra (in Egypt) is almost invariably of the hypochromic normocytic type (Salah, 1935) and the stools do not contain excess of fat.

Addisonian anaemia is unlikely because of the extreme wasting of the patient, the presence of HCl after histamine and the normal icterus index ; besides, the diarrhoea in Addisonian anaemia is not of the steatorrhoeic type.

Accordingly the diagnosis of sprue was finally arrived at. As a matter of fact, the most decisive evidence in favour of sprue was given by the response of the condition to treatment as shown by the return of the stools to normal in regard to number, amount and fat content, the disappearance of the glossitis with return to normal of the previously desquamated papillae, the return to normal of the gastric secretion, decrease in the anaemia and the increase of weight by 21 kg.

TREATMENT.

The line of treatment suggested and reported to be successful by FAIRLEY (1930 and 1932) was followed. The main objects of this treatment are :—

(1) To produce alimentary rest by a suitable diet; BAUMGARTNER and HUBBARD (1927) found that high protein diet gives alimentary rest and does not damage the kidneys in sprue, but because of its high dynamic value it is not suitable for increasing weight. THAYSEN (1932) has pointed out that although fats and carbohydrates are not well tolerated yet the proteins are well absorbed as shown by the finding of a normal nitrogen balance. Accordingly the patient was put on a high protein, low carbohydrate and low fat diet. This regime produced alimentary rest with reduction of motions within 2 weeks to twice daily.

(2) The treatment is directed against the existing anaemia. CASTLE and his co-workers (1935) have shown that the mechanism of production of the macrocytic anaemia of sprue resembled that of Addisonian anaemia, *i.e.* the intrinsic haemopoietic factor is absent in some cases although in others it seems to be the lack of the extrinsic factor which is responsible. In our case the high protein diet alone was without effect on the blood picture. This diet is expected to be haemopoietically effective only when the intrinsic factor is present in the gastric content and absorption from the intestinal tract is adequate. The former could not be verified in our patient owing to the absence of Addisonian anaemia cases to be used as test patients ; the latter is shown to be inadequate by the result of the glucose tolerance test. Marmite, as a representative of the extrinsic factor, was administered in doses of one teaspoonful *t.d.s.* for 12 days without effect on the blood picture.

Liver, 300 grammes *per os*, followed by intramuscular injections of liver extract (2 c.c. campolon and hepractone) for a period of 15 days, was then given. Prompt improvement of the blood picture, tongue and general condition resulted from this treatment as shown in the chart.

(3) The replacement of demonstrable deficiencies is the third aim of the treatment.

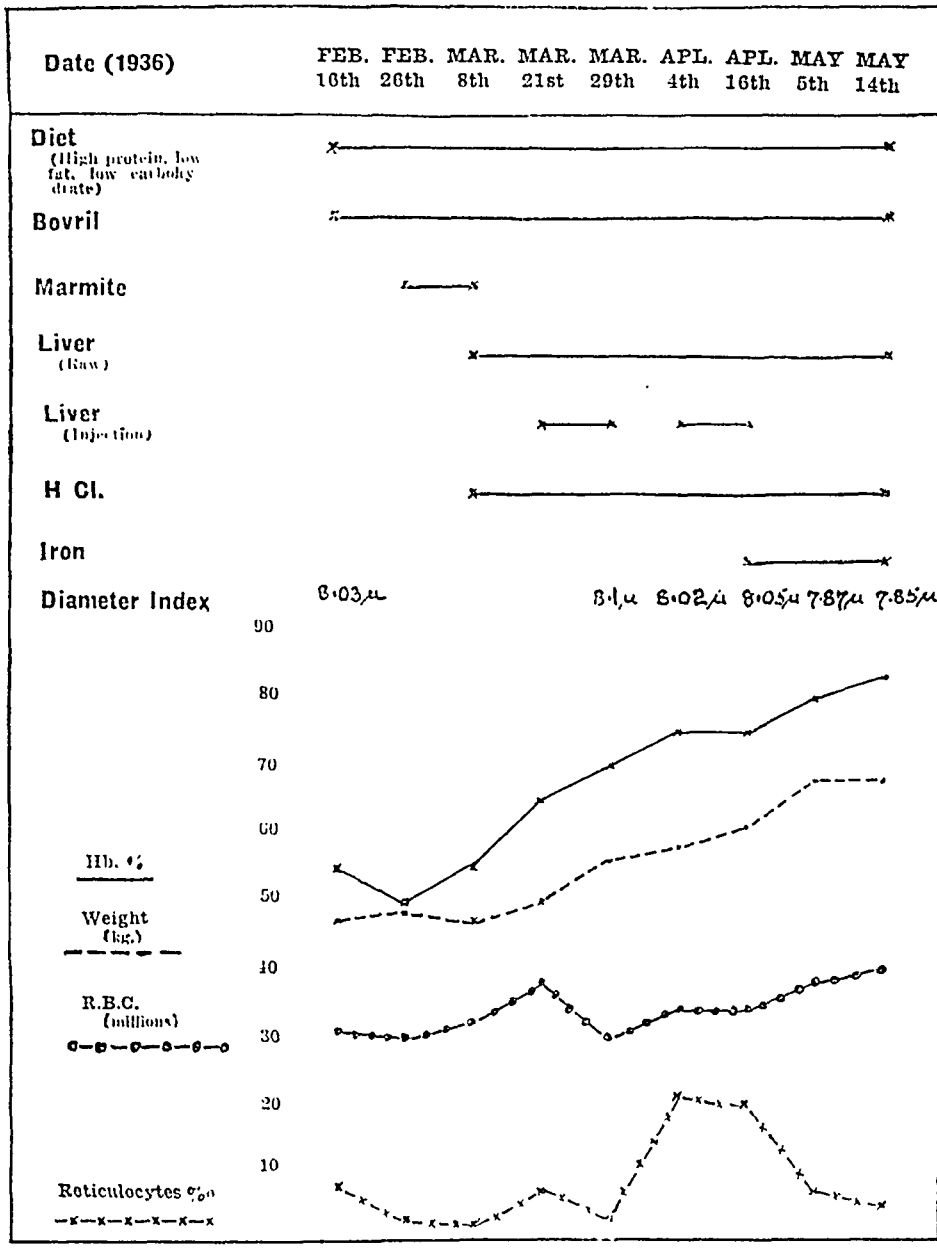
HCl in doses of 4 c.c. in lemonade, *t.d.s.* was given for a period of 2 months. Iron in the form of Blaud's pills, nine daily for 18 days after stopping the liver injections, resulted in a further rise of 5 per cent. haemoglobin.

The improvement became noticeable within 2 weeks of beginning the treatment and reached a maximum at the end of 3 months. (See chart, p. 355.)

A comparison of the various findings on admission, on discharge and also 14 months later is found in the table on page 357.

COMMENT.

Although no cases of sprue have ever been recorded in Egypt the case described in this paper represents the condition in an Egyptian. The diagnosis



of this case was based on clinical, biochemical and therapeutic evidence as well as on exclusion of other conditions giving rise to similar pictures.

Clinically the case demonstrates the glossitis, steatorrhoea, wasting and anaemia which characterize the well-developed condition. The anaemia was of the hypochromic, macrocytic type with a hypoplastic bone-marrow of megaloblastic character suggesting deficiency of both iron and haemopoietic principle.

The abdomen, although sunken on admission, showed the peculiar distension when the number of motions decreased. The liver was clinically smaller than normal; a sign which was reported by MANSON-BAHR (1930) as occurring in about 50 per cent. of sprue cases.

With the exception of diminished deep reflexes there were no signs to suggest implication of the nervous system.

Radiological investigations of the alimentary canal, chest and bones excluded organic lesions and manifestations of any other morbid conditions that might give rise to a similar combination of symptoms (Hodgkin's disease, carcinoma, abdominal tuberculosis, gastro-colic fistula, etc.).

Biochemically the case showed (*a*) achlorhydria with the alcohol meal, HCl appearing only after histamine; (*b*) low blood sugar curve after glucose administration suggesting deficient intestinal absorption; (*c*) normal icterus index; (*d*) chemical analysis of the stools showed marked excess of fat with preponderance of split fat suggestive of steatorrhoea resulting from deficient absorption and excluding pancreatic disease; (*e*) serum calcium value was not reduced although it was at a low normal level.

These biochemical findings are peculiar to sprue.

Therapeutically the case showed marked and complete improvement of all its features under the treatment given and known to cure cases of sprue. This treatment consisted of (1) high protein, low fat, low carbohydrate diet; (2) liver by mouth and by injection, and (3) iron and HCl.

The effects of this treatment were :—

(*a*) An increase in weight of 21 kg. within a period of 3 months, although the character of the diet does not as a rule favour such considerable weight increase. This result is peculiar to sprue.

(*b*) The gastro-intestinal functions returned to normal as shown by the return of HCl secretion; improvement of the glucose tolerance curve; and return of stools to normal in number, character and chemical composition.

(*c*) Disappearance of the glossitis with return to normal of the previously desquamated papillae.

(*d*) Decrease in the anaemia following liver therapy, the haemoglobin rising to 83 per cent. and the red blood corpuscles to 4,875,000 within about 7 weeks. A slight further response occurred after administration of iron. Despite this marked improvement of the blood picture under liver therapy the reticulocytic response was but slight. The same lack of a good response was also reported by CASTLE *et al* (1935) in some of their cases.

The bone-marrow in our case was hypoplastic rather than hyperplastic. ASHFORD (1932) also found cases with a plastic histological picture in specimens of tibial marrow removed during life. It may be that this marrow hypoplasia was responsible for the low reticulocytic response in our case as well as in similar cases reported by others.

The bone-marrow picture returned to normal (erythro-normoblastic) in the course of treatment.

TABLE

Investigations.	On Admission.	On Discharge.	After 14 Months.
Gastric secretion	Achlorhydria, Histamine positive	Normal	—
Stools	Total fat, 60% ; fatty acids, 58·9 % ; neutral fat, 1·1%	Total fat, 45% ; fatty acids, 40% ; neutral fat, 5%	Normal by naked eye and microscopical examination
Glucose tolerance	71/87/100/95/75	77/90/118/100/80	—
Blood picture	Hb. 55% ; R. B. C. 3,110,000 ; Ret. 0·8% ; Diam. Ind. 8·03 ; W.B.C. 3,600; E. 1% ; N. 58% ; L. 36% ; Mono. 3%	Hb. 83% ; R.B.C. 4,875,000 ; Ret. 0·5% ; Diam. Ind. 7·87 ; W.B.C. 6,000 ; E. 9% ; N. 54% ; L. 32% ; Mono. 5%	Hb. 90% ; R.B.C. 4,625,000; Ret. 1% ; Diam. Ind. 7·66; W.B.C. 6,000; E. 5% ; N. 63% ; L. 25% ; Mono. 7%
Bone-marrow sternal puncture)	Hypoplastic, megaloblastic	Normoblastic	—
Icterus index	3 units	3 units	—
Calcium	9 mg. %	9·3 mg. %	—
Weight	47·5 kg.	68 kg.	67 kg.
Tongue	Glazed, red and fissured	Normal	Normal
Motions	4-5 daily	Once daily	Once daily

The patient was discharged in a nearly normal condition and advised to resume his normal diet gradually. This change in diet did not produce any relapse. The case was kept under observation for 14 months after discharge, during which time he remained normal. Some of the findings at the end of this period of observation are reported in the table. The long period of observation in this case has made it possible to exclude certain organic diseases (abdominal Hodgkin's disease, abdominal tuberculosis, etc.) which are known to be able to present for some time a clinical picture simulating sprue (FAIRLEY, 1937).

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

A SUSPECTED CASE OF NON-EPIDEMIC TYPHUS IN A CHILD IN TRINIDAD.

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Pathologist, Trinidad.

As far as can be discovered from the records available locally no case of epidemic typhus has been reported in Trinidad for many years, nor have any cases of typhus-like fevers such as have been found in many tropical countries in recent years. In countries having constant communication with the island, recognised forms of non-epidemic typhus occur such as Brill's disease of the United States and tarbardillo in Mexico. Although the diagnosis was not absolutely proved the following case is thought worth recording if only to stimulate search for possible cases of fever due to this group of diseases in the West Indies. Though evidence of human infection has not been recorded here, the existence of the virus in wild rats in Port of Spain has been proved by the Government Bacteriologist (PAWAN, 1935, verbal communication).

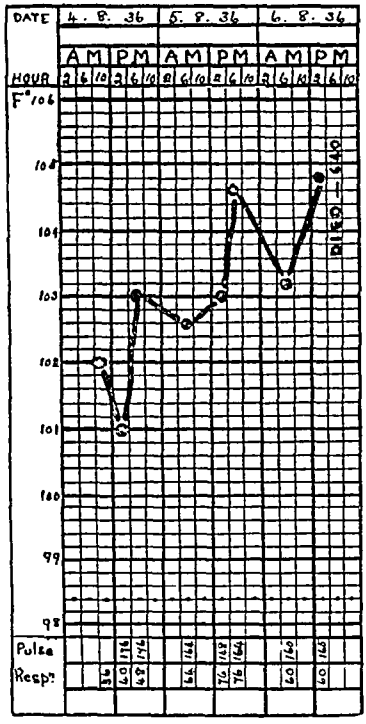
CASE HISTORY.

S. C., an African female child, born in Trinidad, age 4 months, was admitted to the Colonial Hospital, Port of Spain, on 6th September, 1936, with a history of having had fever for about a week and having developed a generalized rash.

On admission, the child was very ill, with a temperature of 101·6° F., a generalized haemorrhagic rash over the body, thickest on the thighs, both hands cold, with marked cyanosis of the left finger tips, and less of the right; the motions were green and offensive.

While in hospital the child had a continuous rising temperature, was very weak and showed evidence of toxaemia, the fingers of both hands became gangrenous, the motions became normal in appearance and death took place 58 hours after admission.

Postmortem Examination.—Unfortunately the parents refused permission for a routine examination, and delay in the internal examination occurred while a certificate was refused and an order obtained for the examination which was eventually carried out 19 hours after death.



The body was that of a moderately nourished female negro infant; the rash was still visible, although faint; discrete spots were present chiefly on the body and especially on the thighs, very few being present on the face; there was dry gangrene of the tips of the fingers of the left hand, with gangrene of the skin extending half way up the forearm, moist gangrene of the fingers of the right hand with the skin peeling off half way up the forearm, the gangrenous skin sharply demarcated from the healthy skin above, the eyes and fontanelles were sunken and the face very toxaemic in appearance.

Thorax.—There was marked hypertrophy of the anterior descending coronary artery, no other abnormality in the heart, the muscle of which was

pale and soft. There were patches of haemorrhagic consolidation in the lungs, especially in the lower lobes, the pharynx was very injected and the cervical glands red and swollen, the thymus was small.

Abdomen.—The liver was swollen and showed patchy congestion, the kidneys were congested with haemorrhages in the left kidney, the pulp in the rather soft spleen was grey with clear Malpighian bodies. The stomach contained undigested milk and the Peyer's patches were conspicuous and the mesenteric glands soft and purple.

Head.—There was oedema of the pale brain, fluid blood in all cranial sinuses and both middle ears were dry and clean.

Laboratory Investigations.

Blood serum (obtained by heart puncture postmortem) agglutination.

	1/10	1/20	1/40	1/80	1/100
Bacillus proteus HX19	++	++	++	+	—
,, ,, OX19	++	++	++	++	—
,, ,, X2	++	++	++	+	
,, ,, XK	—	—	—	—	—

Wassermann reaction, weakly positive ; Kahn, negative.

Animal inoculation.—Intraperitoneal inoculation of a guineapig with brain emulsion had no effect.

Discussion.

This case presented considerable difficulty in diagnosis, no other members of the child's family were, or had been, ill, and no cases of infectious disease likely to give rise to such serious symptoms were present at the time. The possibility of typhus was suggested by the character of the rash and especially the complication of gangrene of the hands, a recognized occurrence in serious cases. It was felt that the titre of agglutinins in the blood for *B. proteus* X19 was not high enough to justify the diagnosis of epidemic typhus in an area completely free from the disease and in the absence of any evidence of the presence of lice on the child or in its home. This opinion has been confirmed by the absence of any other similar cases. The severity of the case was also striking, since epidemic typhus is often a mild disease in small children.

However, the appearance of agglutinins for both *B. proteus* X19 and X2 and the absence of those for the Kingsbury strain in the blood appears suspicious of infection with a fever of the typhus group.

Felix* states that complete agglutination of the O type of 1/80 to 1/100 are significant for diagnosis ; and that dilutions of 1/25 to 1/50 while not

*Felix, A. (1930). *A System of Bacteriology*, vol. 7, 415. London : Medical Research Council.

decisive should not be ignored. The strains used, from the National Collection of Type Cultures, have shown no agglutination with other sera before or subsequent to this case.

In the absence of a scrotal reaction in the inoculated guineapig a certain diagnosis of the case is doubtful, although this may have been due to the dose of virus being small or to the time elapsing between death and the removal of the brain for injection; but it seems possible that this was really a case of an unusually severe infection with non-epidemic typhus, possibly flea-borne.

Summary.

A case of fatal illness with a typhus-like rash and gangrene of the hands in a child is reported, with agglutinins in the blood postmortem in low titre for both *B. proteus* X19 and *B. proteus* X2, but none for the Kingsbury strain.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 3. November, 1937.

TYPHOID AGGLUTININS IN THE NATIVE POPULATION.

BY

R. M. DOWDESWELL, B.CH., M.R.C.S., L.R.C.P.

*Medical Research Laboratory, Nairobi.**

Between December, 1936 and July, 1937, 400 sera from the native population of Kenya Colony sent in for the Kahn test were examined for the presence of agglutinins demonstrable with " H " and " O " suspensions of *Salmonella typhi.* No examination was made for the presence of Vi antibody.

Technique.—The agglutination tubes used had an internal diameter of about 6 mm., the total quantity of suspension and serum dilution was just under 1 c.c. measured by the

*This paper is published by permission of the Hon. the Director of Medical Services, Kenya.

The writer wishes to thank Dr. G. L. TIMMS of this Laboratory for the supply of sera.

drop method.† The tubes, after mixing of suspension nd serum dilution, were kept in a water bath at 52° C. for 2 hours, the water being about one-third the way up the column of fluid and readings were taken after 15 minutes at room temperature. The " H " antigen was a broth culture of a locally isolated smooth strain, showing motility, of 20 hours incubation preserved with 0·2 per cent. formalin and the " O " antigen was made up by diluting to 1 in 10 a 33 per cent. alcoholic suspension, prepared according to Bien's method. Normal saline was used as the diluting fluid throughout. These suspensions were standardized with those provided by the Oxford Standard Laboratories and a suspension factor obtained for them. For instance, if the end point of a given serum with our suspension was 1/500 and corresponded to that of 1/250 with the Oxford suspension having a factor of x, the S.F. of our suspension was recorded as 2x. The sensitivity of the suspensions used has, in fact, been very close to that of the standard ones. In the majority of cases, the sera had been heated to 56° C. for half an hour, but it was found by repeated tests that this procedure affected the agglutination only very slightly, causing a reduction in titre of not more than one-tenth for both " H " and " O," that is a titre of 1/100 might after heating become 1/90 ; so that the results were not significantly affected.

The end point was taken throughout as a trace of agglutination visible to the naked eye [indicated as + according to the conventions used in the international experiment published by GARDNER (1937)].

A preliminary test with serum dilutions of 1/25, 1/50 and 1/150 was first done and all positive sera were then tested again over an appropriate range of finely graded dilutions, with saline controls. No sera showing marked haemolysis were used.

The results obtained giving unreduced titres were as follows :—

TOTAL NUMBER 400—TOTAL NEGATIVES 214.

Observed Titres.	Negative.	1/25 to 1/50	1/50 to 1/100	1/100 to 1/250	1/250 to 1/1,000	Over 1/1,000	Total Positive.
" H " agglutination	291	44	34	17	11	3	109
" O " allutination	257	76	43	20	4		143
" O " agglutination with the absence of " H " agglutination		47	21	8	1		77
" H " agglutination with the absence of " O " agglutination		21	15	4	3		43

It will be seen that there were therefore 16 per cent. positive for " H " agglutinins in over 1/50, and 3·5 per cent. positive in over 1/250.

For all " O " agglutinations there were 17 per cent. positive in over 1/50 and 7½ per cent. of the total showed a titre of over 1/50 for " O " agglutinins in the absence of " H " agglutinins.

†*Note.*—It was found by a number of trials that the use of twice the quantity of suspension and serum dilution here used, in tubes of internal diameter 8·5 mm., gave readings precisely similar to those recorded by this technique.

These results expressed as reduced titres are, therefore :—

Reduced Titres.	0 to 10	10 to 25	25 to 50	50 to 100	100 to 250	250 to 1,000	Over 1,000	Total.	Percentage Positive.
" H " agglutination	—	33	37	19	11	7	2	109	27·25
" O " agglutination	129	10	2	2	—	—	—	143	35·75
" O " agglutination alone	72	4	0	1				77	19·25
" H " agglutination alone		17	18	5	1	2		43	10·75

This shows 9·75 per cent. positive for " H " in a reduced titre of over 50, including 2 per cent. which gave no " O " agglutination : also 3·5 per cent. positive for " O " in a reduced titre of over 10, including 1·25 per cent. which gave no " H " agglutinations.

It should be pointed out that the method used by GARDNER (1929) for the calculation of R.T., by which he estimates standard agglutination before using the S.F. would, applied here, cause a reduction of the R.T., but by not more than 30 per cent.

The three sera showing the highest titres were possibly from patients in the convalescent period or shortly after a true typhoid infection. Unfortunately, it has not been possible to exclude entirely the possibility of a previous inoculation,* but the percentage of such cases would be very small.

DISCUSSION.

A strict comparison of the results obtained here, with those published by others, is not possible and such an attempt would be misleading. For

*Note.—There is no doubt that inoculation with the phenolized vaccine prepared here will give rise to an appreciable production of " O " antibodies, and apart from a number of examinations after secondary inoculations which have shown unreduced titres for *S. typhi* " O " of over 1/500 and some of over 1/1,000 the only case of a primary inoculation done at the Laboratory on a European adult, whose serum showed no agglutination in dilutions of 1/25 or over before inoculation, gave 10 days after the last injection unreduced titres :—

S. typhi " H "	1/1,250	(S.F. 1·1)	
S. " " O "	1/833	(S.F. 13)	
S. paratyphi " A " " H "	1/500		
S. " " A " " O "	1/125		
S. " " B " " H "	1/1,200		
S. " " B " " O "	1/100		

instance, although GARDNER (1929), GARDNER and STUBBINGTON (1932) and SMITH (1932) show results as reduced titres ; others as ALVES (1936) and BEATTIE and ELLIOT (1937) make use of standardized suspensions from the Oxford Laboratories but do not refer to the suspension factors ; while some as GRASSET and LEWIN (1936), GIGLIOLI (1933), HORGAN (1932) and DENNIS and BERBERIAN (1934) do not appear to have standardized their suspensions. Further anomalies may be caused by differences in technique, such as reading of end points : anomalies only too clearly demonstrated in the international experiment already quoted. However, a few results are quoted below for rough comparison :—

RESULTS OBTAINED WITH "NORMAL" SERA.

"H" Agglutination.

Author.	Material.	No. of Sera.	Result.
GIGLIOLI (1933)	Population, British Guiana	350	34·2 per cent. positive in dilutions of over 1/10 16 per cent. positive in dilutions of over 1/40
HORGAN (1932)	Natives of Sudan	70	0 per cent.
WHITEHEAD (1930)	British Army	67	..
BEATTIE and ELLIOT (1937)	British	47	..
ALVES (1936)	Natives, Southern Rhodesia	530	12·6 per cent. positive in dilutions from 1/50 to 1/500

"O" Agglutination.

Author.	Material.	No. of Sera.	Result.
GARDNER (1929)	Europeans, England	47	17 per cent. positive between R.T. 1·5 and 4
GARDNER and STUBBINGTON (1932)	Europeans, England	47	38 per cent. positive in an R.T. of less than 3 except one of 15
GIGLIOLI (1933)	Population, British Guiana	350	31·4 per cent. positive in dilutions of 1/10 to 1/80
HORGAN (1932)	Natives of Sudan	70	7 per cent. positive in dilutions of 1/25 and 1/50
WHITEHEAD (1930)	British Army	67	21 per cent. positive in dilutions of 1/25 and 1/50
BEATTIE and ELLIOT (1937)	British	47	13 per cent. positive in dilutions of 1/20 and 1/40
ALVES (1936)	Natives, Southern Rhodesia	300	15 per cent. positive in dilutions of 1/50 to 1/500

In typhoid infection, GARDNER (1929) reports forty cases in which the reduced " O " titre varied between 6 and 114. SMITH in Scotland found no reduced " O " titres of over 100 in twenty-eight cases; HORGAN apparently obtained low titres from 0 to 1/500 in fourteen cases except one previously inoculated who gave 1/2,500. From a very small series of observations here, where of forty consecutive sera sent for Widal examination, of which only eleven showed an " H " titre of more than 1/50 for T., A. or B., these eleven with typhoid " H " titres of from 1/250 to 1/6,000 gave reduced titres for " O " of 275, 2, 115, 270, 641, 192, 77, 77, 192, 6 and 64. A further series is contemplated, but it is, however, suggested that the presence of such a considerable percentage of " O " agglutinins in the " normal " native population although in very low titre may be correlated with the production of unusually high titres in disease.

CONCLUSIONS.

1. The results of the examination of 400 sera of natives, not suspected of typhoid fever, for the " H " and " O " agglutinins of *S. typhi* are given.

2. That the high incidence of both " H " and " O " agglutinins suggests the presence among the population of a considerable amount of typhoid infection.

3. The finding of " O " agglutinins alone suggests previous infections overt or latent, possibly by other members, having " O " antigen IX, XII, of the *Salmonella* according to the KAUFFMANN-WHITE schema (1934).

REFERENCES.

ALVES, W. D. (1936). *S. Afr. med. J.*, **10**, 6 and 7.
BEATTIE, C. P. & ELLIOT, J. S. (1937). *J. Hyg. Camb.*, **37**, 36.
DENNIS, E. W. & BERBERIAN, D. A. (1934). *Amer. J. Hyg.*, **20**, 469.
GARDNER, A. D. (1929). *J. Hyg. Camb.*, **28**, 376.
———. (1937). *Ibid.*, **37**, 124.
GARDNER, A. D. & STUBBINGTON, E. F. (1932). *Ibid.*, **32**, 516.
GIGLIOLI, G. (1933). *Ibid.*, **33**, 379.
GRASSET, E. & LEWIN, W. (1936). *Brit. J. exp. Path.*, **17**, 179.
HORGAN, E. S. (1932). *J. Hyg. Camb.*, **32**, 523.
KAUFFMANN, F. & WHITE, P. E. (1934). *Ibid.*, **34**, 333.
SMITH, J. (1932). *Ibid.*, **32**, 143.
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WHITEHEAD, N. T. (1930). *J. R. Army med. Cps.*, 55, 81.

CORRESPONDENCE.

BANCROFTIAN FILARIASIS AND THE RETICULO-ENDOTHELIAL SYSTEM.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

All those whose paths have now diverged from their original first love—the study of filariasis—a study which must always be regarded as the corner stone of tropical medicine, will find their interest aroused afresh by Colonel CLAYTON LANE'S paper in these TRANSACTIONS.*

There may be some who, like myself, find his arguments, though clearly expressed in faultless English, just a little difficult to follow. The recent advances in knowledge are so many and so diverse in this direction that it is hard to keep pace with them. Into the vexed question of the mechanism of periodicity, I do not propose to enter now, as I am definitely of the opinion that this matter cannot be settled unless many more detailed observations can be obtained and some suitable experimental animal, susceptible to infection with *Wuchereria bancrofti*, discovered. Then, and only then, can this matter be transferred from the *cumuli* of speculation to the *terra firma* of verifiable facts.

But where I feel inclined to disagree with Colonel CLAYTON LANE is where he refers to my own part in the elucidation of the pathology of filariasis. Naturally I feel it a great honour that he should refer to my own work at all, and I feel loathe to enter, at this stage, on any question of priority. But I do maintain that I was the first to demonstrate the complete, and as it has proved to be, accurate pathology of filariasis. This was described by me in the account of my work in Fiji in 1910 and 1911, published in 1912. I feel disposed to claim that my figures and drawings clearly demonstrate that I was the first to show the part played by endothelial cells in the pathology of Bancroftian filariasis. A great deal of the credit for the proper explanation of the pathological picture has been attributed by Colonel CLAYTON LANE to my friend, the late Professor F. W. O'CONNOR. It is true, I admit, that his material was much more extensive and much more carefully worked out than in the case of the scantier and hastily collected material I was able to obtain under much more primitive conditions and with less extensive assistance; but I do claim that the main facts have been borne out by more recent work. I cannot and do not claim that it was proved that the dead and defunct microfilariae

*LANE, CLAYTON. (1937). Bancroftian filariasis and the reticulo-endothelial system. *Trans. R. Soc. trop. Med. & Hyg.*, **31**, 61.

are engulfed by endothelial cells, but I was able to demonstrate proliferation of the endothelial lining of lymphatic channels, giant cell formation and proliferation of fibroblasts in glands from which adult filariae were obtained. In mentioning the fact (p. 62) that CRUICKSHANK and WRIGHT (1914)* demonstrated the formation of giant cells in the vicinity of adult filaria parasites, my previous work mentioned above appears to have been overlooked. My figures (Plate XXV., Figs. 1, 2 and 3)† abundantly prove that I had already, in 1912, described and figured this giant cell formation.

No one more than myself is ready to confess that many gaps existed in the completeness of my work on filariasis and elephantiasis in Fiji, but even at this distance of time I think that what I was able to show was the best that could have been done under the circumstances.

I am, etc.,

PHILIP MANSON-BAHR.

BEJEL.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

DEAR SIR,

I have read with very great interest the paper by Dr. E. H. HUDSON‡ on bejel and the discussion thereon. Dr. HUDSON has described a disease existing under this name amongst a relatively small Bedouin population of the Euphrates valley, whilst Dr. CORNER has seen the same disease under the same name in the Tigris valley. Dr. HUDSON has suggested that the disease presents a sufficiently distinct clinical and epidemiological entity to justify the inclusion of the word " bejel " in medical nomenclature.

Throughout much of the Sudan, " bagl " (" g " usually soft) is a well-known disease. There is no doubt that " bejel " and " bagl " are transliterations from the same Arabic word. The difference is but one of phonetic convention.

In the Sudan, however, " bagl " is a colloquial word with the sole meaning of gonorrhoea. Unlike the British clinicians before the day of JOHN HUNTER'S classic experiment, the Sudanese make a clear distinction between gonorrhoea and syphilis and they are aware of the generally venereal origin of both diseases.

I am informed by Dr. R. H. KHABBAZ, a graduate of Beirut, that the word " bagl " or " bejel " is not used in Syria to mean either syphilis or gonorrhoea. I understand that the word is not used in Egypt. It is not given in *An English-Arabic Dictionary of Medicine, etc.*, by Dr. MOHAMAD SHARAF (published by the Ministry of Education, Egypt).

*CRUICKSHANK, J. A. & WRIGHT, R. E. (1914). *Indian J. Med. Res.*, 1, 741.

†BAHR, P. H. (1912). *J. Lond. Sch. trop. Med.*, Supplement No. 1.

‡HUDSON, E. H. (1937). Bejel : the endemic syphilis of the Euphrates Arab. *Trans. R. Soc. trop. Med. Hyg.*, 30 (1), 9.

Through the kindness of Mr. TEWFIK KHABBAZ, a scholar of Arabic, I learn that " baglah " is an obsolete Arabic word meaning ulcer. Of this word, " bagl " is a plural form. Dr. HUDSON'S patients are thus, apparently, complaining of ulcers. The singular form of " bagl " is occasionally used in the Sudan meaning gonorrhoea.

It is generally held that the Arabic penetration into the Sudan came originally from the East. It is striking that the word transliterated " bejel " or " bagl " has survived in two districts relatively remote and has apparently not survived in the associated countries of Syria and Egypt. I do not know whether it is used or not in other parts of the Arabic-speaking world. It is furthermore interesting to speculate as to the different meanings, venereal and non-venereal, which the word has acquired.

Dr. HUDSON has pointed out that gonorrhoea is rare amongst the Bedouin Arabs of the Euphrates. It would interest me to learn the name they give to this disease and whether they realize its venereal origin.

Finally, it remains to raise a mild protest against the incorporation of " bejel," meaning non-venereally acquired endemic syphilis, in medical nomenclature. We, and our several million Sudanese patients, shall continue to look upon " bagl " as a generally venereally acquired disease—gonorrhoea.

I am, etc.,

Mudiria, Khartoum,
Sudan.

H. RICHARDS.

ELECTION OF HONORARY FELLOW.

PROFESSOR BERNHARD NOCHT.

At the Council Meeting on 21st October, 1937, reference was made to the forthcoming celebrations in Hamburg on 4th November, in connection with the eightieth birthday of the veteran Professor BERNHARD NOCHT.

In recognition of the great importance and international value of his work in tropical medicine, it was unanimously decided to make Professor NOCHT an HONORARY FELLOW of the Royal Society of Tropical Medicine and Hygiene, and to send to him the congratulations of the Council on the occasion of his eightieth birthday.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE.

VOL. XXXI. No. 4. JANUARY, 1938.

Proceedings of a **Clinical** and **Laboratory Meeting of the Society,** held at the Hospital for Tropical Diseases, Gordon Street, London, at 8.15 p.m., on Thursday, 18th November, 1937.

Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.), *President*, in the Chair.

DEMONSTRATIONS.

Mr. A. H. McIndoe and Dr. P. Manson-Bahr.

Unusual case of elephantiasis of the leg in a woman treated by operation.

The circumference of the thigh measured 36 inches, that of the leg 32 inches. The elephantoid condition was undoubtedly of streptococcal origin and its

development had been associated with pyrexial attacks in which the patient was severely ill. All sources of focal sepsis were eliminated prior to operation, the mouth being cleaned and all septic teeth extracted. Operation consisted in the removal of a considerable quantity of skin and subcutaneous tissue from the medial aspects of leg and thigh.

Dr. P. Manson-Bahr.

Cases :

(1) Amoebic infection of skin surrounding a colostomy wound (photograph and sections).

(2) Amoebic ulcer of the rectum of 20 years' standing, resembling carcinoma.

(3) Two cases of abortus fever.

(4) A case of acute dilatation of the stomach in amoebic dysentery.

(5) Case of pancreatic cyst—diagnosed primarily as a malarial spleen.

(6) Acute amoebic hepatitis resembling typhoid.

Dr. G. W. M. Findlay.

Two methods of growing viruses for purposes of immunization.

(1) Growth in flasks of serum Tyrode solution containing a small amount of minced chick embryo.

(2) Growth on the chorio-allantoic membrane of the developing chick embryo.

Mr. W. H. McMullen and Major J. A. Cruickshank.

A case of *Filaria bancrofti* in the interior of the eye.

Only two certain cases have been reported previously, one by KOMAN NAYAR and one by R. E. WRIGHT. The present case is an Indian student from Calcutta. He has microfilariae in the blood and an eosinophilia of 27 per cent., but he shows no clinical signs of filariasis.

He gives a history of iritis a year ago and the present attack of iritis commenced in September. It presented no special features at first, and was gradually clearing up. On 6th October during examination with the slit lamp, there suddenly came into sight in the aqueous a minute worm-like object, actively motile and very thin. Its extremely sinuous form and active motility made estimation of length very difficult. It was seen only for a few seconds and apparently disappeared behind the iris. It was seen again on 13th October, and shown to Colonel WRIGHT who agreed that the object seen in the anterior

chamber was undoubtedly a nematode, a very young one, much smaller than those he had seen previously in the eye in India.

Dr. G. R. Mather Cordiner.

A series of radiograms showing appearances in ulcerative colitis.

Dr. A. F. Cole.

X-ray photographs of a case of extensive necrosis of the cranial bones following empyema in a boy aged 17½.

May, 1936.—Pleurisy followed by empyema. *June*, 1936.—Rib resection and siphon drainage: contents 3 pints pus. No tubercle bacilli but anaerobic streptococci present. Healed completely. *November*, 1936.—Slight blow to occiput was followed by necrosis of occipital bone. Anaerobic streptococci present.

Subsequently there has been progressive necrosis of both parietals, both temporals, and both frontal bones. Many sequestra have been removed and seven drainage incisions made. Necrosis steadily advancing.

Treatment.—6 ferrous sulphate pills (Glaxo) daily; 3 prontosil tablets daily; open air and good feeding; peroxide of hydrogen lavage.

Present Blood Picture, *November*, 1937.—Haemoglobin, 78 per cent. Reds, 5,080,000 per c.cm. Whites, 13,800 per c.cm. Colour index, 0·78. Differential count: Polymorphs, 80 per cent.; lymphocytes, 14 per cent.; monocytes, 6 per cent. No abnormal red cells. Wassermann negative.

Summary.

It is difficult to decide whether to advise extensive necrosis operations or to continue to encourage natural resistance with local drainage and drugs.

The periodic examination of pus films shows remarkable decrease in numbers of streptococci: on one occasion two out of seven cultures from different sinuses remained sterile and it is possible that prontosil may have been a factor in producing this result.

Dr. N. Hamilton Fairley.

I. Pathological specimens in recent fatal cases of Weil's disease.
II. Cases.

(1) Hepatic cirrhosis with haematemesis and megalocytic anaemia (storage defect ?).

(2) Tropical sprue treated with anahaemin—Haematological response.

(3) Case with calcification of the spleen of unknown aetiology (X-ray picture).

Dr. N. Hamilton Fairley and Mr. Naunton Morgan.

Case.

Segmental ulcerative colitis with polyposis (X-ray pictures).

Dr. N. Hamilton Fairley and Mr. R. J. Bromfield.

Pseudo-methaemoglobin, occurring naturally in pancreatic cyst fluid as well as that produced artificially was demonstrated on the Hartridge reversion spectroscope. Tables were shown comparing and contrasting pseudo-methaemoglobin with other blood pigments such as methaemoglobin and sulphaemoglobin having an α band in the red portion of the spectrum.

I. Pseudo-methaemoglobin—its demonstration in pancreatic cyst fluid.

Over 3 litres of dark brown turbid fluid was aspirated by Mr. McINDOE from a large pancreatic cyst in a patient under the care of Dr. MANSON-BAHR. The fluid, which was just alkaline to litmus, contained albumin and diastase, but no bile salts or pigments. The benzidine test was positive. After centrifugalization an occasional red cell and leucocyte were found in the deposit.

Spectroscopical examination showed no oxyhaemoglobin but an α band in the red at approximately 6230 Å, with a diffuse absorption in the green-blue portion of the spectrum. The α band remained unaltered after treatment with (1) freshly prepared Stokes's reagent; (2) ammonium sulphide (10 per cent.); (3) hydrogen peroxide (10 volumes); (4) coal gas; (5) hydrazine hydrate (50 per cent.). On the other hand, it was definitely dispersed by solid sodium hydrosulphite ($Na_2S_2O_4$). In its behaviour to chemical reagents the pigment corresponded to pseudo-methaemoglobin. Pseudo-methaemoglobin can be readily distinguished from methaemoglobin and sulphaemoglobin by carrying out the above-mentioned tests, as well as by comparison on a Hartridge reversion spectroscope, as was demonstrated at the meeting. The α band of pseudo-methaemoglobin was shown to lie mid-way between methaemoglobin (6300 Å) and sulphaemoglobin (6180 Å). With methaemoglobin the α band was dispersed with all six reagents mentioned. With sulphaemoglobin, though the α band was slowly dispersed with hydrogen peroxide and showed a slight shift towards the green with coal gas, it remained unaltered with Stokes's reagent, ammonium sulphide (10 per cent.), sodium hydrosulphite and hydrazine hydrate (50 per cent.).

Comment.—In a former communication* to the Society we have shown that pseudo-methaemoglobin is produced in severe intravascular haemolysis such as occurs in blackwater fever and incompatible transfusion where oxyhaemoglobin is liberated from the corpuscles into the circulating plasma.

*FAIRLEY, N. H. & BROMFIELD, R. J. (1937). Pseudo-methaemoglobin in blackwater fever and its clinical significance. *Trans. R. Soc. trop. Med. Hyg.*, **31**, 139.

The present finding is of importance in as much as it shows that pseudo-methaemoglobin may also be produced outside the vascular system when blood escapes into a cavity, is haemolysed and subsequently mixes with plasma or serous transudate or exudate. We predict its presence in the brown coloured fluids found in other types of cysts which have contained blood as well as in joint effusions occurring in the haemarthroses.

II. Artificial production of pseudo-methaemoglobin

(*a*) *By incubating Solutions of Oxyhaemoglobin with Plasma derived from certain Animal Species.*

For this purpose plasma derived from man and monkeys of the species *Macacus rhesus*, *M. iris* and *Cercopithecus aethiops* were utilised. When 1 volume of a strong solution of oxyhaemoglobin from any of these sources was incubated at 40° C. with 3 volumes of human or monkey plasma collected under sterile conditions, pseudo-methaemoglobin was produced within a period of 2 to 3 days. On the other hand, when solutions of oxyhaemoglobin were incubated with plasma derived from laboratory animals such as the rabbit, guineapig and rat, pseudo-methaemoglobin was never demonstrated, though methaemoglobin appeared in certain of the specimens. Control solutions of oxyhaemoglobin from all species readily produced methaemoglobin when incubated alone for a period of 12 to 24 hours.

(*b*) *By adding Alkaline Haematin to Plasma derived from certain Animal Species.*

Alkaline haematin was prepared by adding pure haemin to water made alkaline with two or three drops of 10 per cent. sodium hydroxide, and this was subsequently added to plasma derived from man, *Macacus rhesus*, *M. iris* and *Cercopithecus aethiops* and certain other animals including the rabbit and guineapig.

In the case of human and monkey plasma pseudo-methaemoglobin was immediately produced, but not in that of any of the other animals investigated.

Comment.—The spectroscopic picture of pseudo-methaemoglobin produced by incubating oxyhaemoglobin with plasma at 40° C. was identical with that encountered in blackwater fever, the β and γ bands closely approximating to those of oxyhaemoglobin. When the pigment was synthetised by adding pure alkaline haematin to appropriate human or simian plasma known to be spectroscopically free from oxyhaemoglobin, the α bands closely approximated or were co-linear, but the β and γ bands were no longer demonstrable, being replaced by a general absorption in this portion of the spectrum.

Apart from these different spectroscopical features, which appear to be dependent on the absence of oxyhaemoglobin in solutions of the synthetised pigment, the chemical reactions of the incubated and synthetised pigment are similar in all respects.

From these and other observations there can be little doubt that pseudo-methaemoglobin is a by-product of haemoglobin katabolism, and is formed both *in vivo* and *in vitro* by the union of haematin with some protein or other nitrogenous constituent contained in human and simian plasma. The observation that the plasma of laboratory animals is incapable of producing pseudo-methaemoglobin either on prolonged incubation with strong solutions of oxyhaemoglobin at 40° C. or on the direct addition of alkaline haematin, indicates that the particular protein or nitrogenous constituent implicated is either absent or present in insufficient concentration to form the new pigment in demonstrable amounts. On the other hand, the oxyhaemoglobin, derived from any species of animal, is equally effective as a source of pseudo-methaemoglobin. This, however, is not surprising since the haemoglobin of mammals varies in the globin and not in the haematin content of the molecule.

From the physiological and pathological viewpoints, these observations imply that pseudo-methaemoglobin is only likely to be formed in man and monkeys, and not in ordinary laboratory animals. This is probably one of the reasons why pseudo-methaemoglobin has not been previously recognised by laboratory workers.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

Proceedings of an **Ordinary Meeting** of the Society, held at Manson House, 26, Portland Place, London, at 8.15 p.m., on Thursday, 9th December, 1937.

Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.), *President*, in the Chair.

PAPERS.

THE EPIDEMIOLOGY AND CONTROL OF LEPROSY.*

BY

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Either of the two subjects mentioned in the title of this paper might, in its complexity and far-reaching scope, be worthy of a treatise, I shall therefore now study the epidemiology of leprosy only briefly and in so far as is necessary to suggest methods of controlling the disease.

Epidemiology is a word which has to be justified when applied to leprosy. We think of epidemics in connection with acute diseases introduced from without with a more or less rapid rise and fall of incidence and virulence; or as related to a temporary increase in an endemic disease. What do we know of the introduction of leprosy? We read of leprosy in India in the Vedic times, and of leprous slaves being imported from the sources of the Nile into Egypt several thousands of years before Christ; but all is so vague and so distant in time and space that it is difficult to associate leprosy with a graphic curve. Leprosy, as found in the great continents of Asia and Africa, seems like the surface of the ocean, almost a dead level, stretched out over the centuries from the time of prehistoric man.

It is when we find leprosy introduced in recent times into islands where it

*This paper was read previously at the Edinburgh Branch of the Royal Society of Tropical Medicine and Hygiene on 25th October, 1937.

did not previously exist, and rapidly spreading through the native population; and where, as in the Island of Nauru, in the Western Pacific, modern methods of control have checked and are gradually eliminating the disease, that we can see clearly that leprosy is subject to the laws of epidemiology.

Whatever the actual causes which control the spread of leprosy, it seems clear that it belongs to a certain stage of human social development. In India it is not found among the nomadic tribes or among the aboriginals until they forsake their tribal customs and mix with, and adopt the customs of, more civilized people. In Arabia and in the Sudan in the wild nomadic life of the desert leprosy apparently hardly exists. It is in the comparatively fertile Yemen and at points where the contact with the outer world is most close that the disease is found.

On the other side of the picture we find leprosy constantly being introduced in our own country, by those who have been infected in the tropics; and yet, though it is not a notifiable disease and few special precautions are taken, leprosy with a few notable exceptions, does not spread. Recently some agitation was caused in France because of the ninety-five known lepers in Paris. Six had never been outside the country, and had contracted the disease by sexual contact with lepers and four others had contracted it conjugally. It is noteworthy that they were all adults. Perhaps the reason why leprosy does not spread in western Europe (with the exception of the Iberian Peninsula) is that, though it enters the community, it seldom enters the family. Those who settle in this country after contracting leprosy in the tropics seem for various reasons seldom to come into close contact with young children. There is evidence that, apart from the infection of children, leprosy has little tendency to spread.

MOLESWORTH upholds the theory that the present-day exemption of western Europe is due to racial immunity, the more susceptible strains having been eliminated during the Middle Ages, when leprosy was prevalent. There is little evidence, however, to support this theory. It is true that there were once 2,000 lazarets in France, and 300 in this country; but there is reason to believe that many of those formerly called lepers were not suffering from HANSEN'S infection, and that leprosy was never sufficiently prevalent to have considerably immunized the community.

The general statement therefore holds good that leprosy lies in the social stratum between nomadism and a certain moderately high level of sanitary and economic advancement. But even short of a high degree of such advancement there are many factors which have considerable influence in controlling the disease.

Among these factors are the traditional customs regarding leprosy which grow up in the community. Recently reports have appeared of outrages in China, where some 200 lepers are known to have been lined up and shot by military leaders. It is only recently that such occurrences have been widely reported in the Press, but this method of dealing with leprosy is no new thing

in China. Nor is China the only place where such action has been taken. In south-west Nigeria leprosy is comparatively uncommon in spite of its great frequency in the Northern Provinces, and especially in the centre and east of the Southern Provinces. One likely explanation is that in the old slave-trade days lepers were got rid of in a manner similar to that in China. Such methods, apart altogether from their appalling cruelty, often defeat their own object, as they drive likely victims to concealment and consequently favour further spread of the disease.

The same rule holds good regarding all use of compulsion in leprosy, if it is applied from outside the community. This has been well exemplified in the Philippine Islands, where compulsory laws of segregation applied since the beginning of this century have done but little to get to the root of the problem.

The relationship of density of population to leprosy is an interesting one. Unlike many other diseases leprosy is not as a rule associated with a dense population. In Great Britain it lingered on longest in scattered hamlets of Cornwall and Shetland. In Norway it continued in the fishermen's huts in the lonely fjords. In the lonely valleys of the Himalayas it lingers on in little cabins generation after generation. This is probably explained in part at least by the peculiar susceptibility of the child to leprosy, the low toxicity and low fatality of the disease, and the long period after infection before the first signs are recognized. Thus leprosy is a disease of the crowded house, room and bed, and the more crowded and insanitary these are, the more likely is it to spread. In India the joint family system has an important bearing. In the first generation the house may be adequate in size ; but in the second generation, when the families of several sons have to be accommodated in the same building, overcrowding is the result ; and if one leper is introduced the infection has every chance of spreading by close daily contact. In Indian villages, the greater the amount of mixing of all castes and strata of the population, the more does leprosy tend to spread. This is especially so in industrial centres where tribal and caste rules are to a large extent laid aside, and those among whom leprosy is a common disease mix freely with other races who know little of leprosy and are unable to recognize it.

The rise and fall of the leprosy curve in an area is not due to the development of physiological immunity so much as to the growth of a mental and social immunity. As the disease increases in the community its members learn to recognize it and take precautions. These precautions may not be adequate, nor sufficient to eliminate the disease, but they are generally sufficient to curtail it. Often the deformed neural case, in whom infection has more or less died out, will, because of his conspicuous signs, be isolated outside the village ; while the highly infectious cutaneous type is still allowed to mix with all and sundry. The fact that children, though infected in their earliest years, do not show conspicuous signs till puberty, leads to the wrong assumption that they are not susceptible, and they are allowed to mix freely with infectious cases.

The Use of Compulsion.

What then are the general principles which should govern a campaign for the control of leprosy in an endemic area ? Compulsory segregation of all cases in institutions would be the ideal method. This may be possible in small isolated areas like the island of Nauru in the Western Pacific, where autocratic power is effective ; or it might be applied where people are sufficiently intelligent and advanced to lend co-operation. But in vast areas like India, China and Africa the people are ignorant and superstitious, and the incidence is often 1 per cent. of the population, and not infrequently may rise much higher (in West Africa it is 10 to 15 per cent. in many places). There institutional segregation is obviously out of the question except for a certain small fraction of the whole, and compulsory laws enforced by those outside the community would lead to concealment and thus defeat their own end.

Compulsion has its right place, however, when it is applied not from without but from within—by the community itself. In England one of the most important factors in stamping out leprosy was the application of a modified form of the old Jewish law. The certified leper was counted as dead and banished by the community from its midst. The burial mass was pronounced over him and he was forbidden to buy or sell in the market or to come into close contact with others, and especially with children. These measures may not have been very humane, but they were in keeping with the times, and they were apparently effective. They were a result of the translation of the Bible, and were a modified application of the levitical law following on an educational campaign by the Church, which was at that time the chief source of enlightenment and progress in the land.

In some parts of Africa leprosy is proving an alarming problem. In parts of the Belgian Congo the proved incidence is from 10 to 15 per cent., and in some places even more. There seems little doubt that this is largely due to lack of sanitation and the poor physique of the people, suffering severely as they do from parasitic and nutritional diseases. The people realize the danger of contagion, and their clan system would drive out the foreign leper. But family instincts are strong and the child is closely nursed and tended by the infectious parent or relation, and so the disease spreads within the family and clan. Formerly the people were always at war with each other and the clans did not mix. Now, under European control, war is abolished and former enemies fraternize and the conditions for the spread of leprosy are thus more favourable.

Treatment.

The place of treatment in the control of leprosy is an important one, but one which has often been exaggerated and misunderstood. We have no specific treatment for leprosy, any more than we have for tuberculosis. The most we can say is that leprosy compared stage by stage with tuberculosis is somewhat

more amenable to treatment than the latter disease. In both, the main reliance must be placed upon general methods and improvement of the health of the patient. When this can be secured, and with the help of certain special remedies, such as chaulmoogra oil and its preparations, the majority of cases may be expected to recover, though the more the disease advances beyond a certain stage the more difficult and hopeless does it become. But the main difficulty, at least at present, is to secure the general hygienic and nutritional conditions necessary to make special treatment of any avail.

Perhaps the main value of out-patient treatment is as a means of winning contact and the good will of the patient. Once these are secured it is possible to follow him up to his home, and there, after examination of contacts and thorough investigation of social and economic conditions, to institute an educational campaign.

Such a campaign is not by any means confined to leprosy. In fact leprosy may be used as a key to open the door to general hygienic reform. When arbitrary compulsion is attempted the fear of leprosy drives to concealment. But when the patient's co-operation has been won by offering him treatment, and this has been followed up by a careful survey in the village, tracing the spread of the disease through past generations, then the dread of leprosy may be used as a potent factor in inducing the individual and the community to take the simple means necessary to prevent the spread of the disease. For leprosy, though difficult to cure, is easy to prevent when the good will, understanding and co-operation of those concerned have been won.

Leper Settlements.

One of the all-essential factors in the control of leprosy is the large, well-staffed leper settlement. Such an institution must not be confused with the old type of leper asylum, where hopeless cases, driven out from their homes, congregate and pass their miserable and hopeless days fed by charity. The modern leper settlement, some of the best of which may be seen in Nigeria, is a very different place. There the patients are suitably housed and spend their lives in cheerfulness and usefulness. There it is possible to organize treatment in its only effective form—occupational therapy. Such a settlement is a hive of industry; agriculture, horticulture, various industries, house building and road making are among the many activities. School and Church, scouts and guides, orchestra and dancing are among the many educational and social amenities, while the lepers themselves act as chiefs, magistrates and police to maintain order and organize various departments.

The well-run leper settlement is in fact a model village, or cluster of villages —a centre of culture and progress, not only benefitting its own inhabitants but potentially a source of enlightenment and sanitary and social development to the whole area. This is indeed the most important aspect of the leper settlement.

It is a thoroughly democratic institution, and yet discipline and training enter into all its activities. A certain proportion of the patients can look forward to complete recovery, and after their years of training in public health, and especially in the prevention of leprosy, they may take a valuable share in the campaign against leprosy in the villages.

Such an institution requires money, but when well organized it is astonishing how far the money will go. It cannot of course be self supporting. Many of the patients are in excellent general health, and the large majority can do a good day's work, but there is always a residue of the weak and the helpless, the crippled and the blind. In Nigeria the financial burden is largely met by the Government and Native Administrations, but the staff and organization and administration come from various missions. The success of a leper settlement necessarily depends on the man in charge; and highly qualified and devoted men and women have not been wanting to undertake this difficult and laborious task, on salaries little more than a subsistence allowance; all honour to their noble and altruistic spirit.

What of the Future of Leprosy Control?

Leprosy in endemic countries is so inextricably bound up with economic, social, educational and sanitary problems, that its control can only advance simultaneously with the solution of these problems. It is significant that in this country the lazaret was the precursor of the modern hospital, and that many of the early public health laws in this country were passed for the control of leprosy. From its very nature leprosy is qualified to be a key disease, a key to open the door to further general sanitary and social reforms. I have already described the Nigerian leper settlement as a centre of general enlightenment in the district where it is situated. Likewise the fear of leprosy may be used as a force which, if wisely directed, will drive the endangered villager to adopt general sanitary reforms.

Dr. MUIR showed a cinematograph film illustrating the activities of a leper settlement.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

TUBERCULOID LEPROSY.

BY

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INTRODUCTION.

There is no other aspect of leprosy which has attracted so much attention in recent years as the tuberculoid lesion. The recognition of this variety of leprosy is probably the most important advance in the study of the disease since HANSEN discovered the leprosy bacillus in 1874. After the discovery of hydnocarpus oil attention was almost exclusively devoted to the application of its soaps and esters in the treatment of leprosy. In India especially the drug was hailed as a specific, and adverse reports from other countries scarcely tempered the enthusiasm of the Indian leprologists. In any case the circumstances were peculiar. The fact is that hydnocarpus *was* producing results in India even though it was not so successful elsewhere. Again, outside India, doctors were reporting trials on series of patients with discrete lesions which could be accurately described, measured and, above all, photographed before and after treatment. In such a series of cases the results of treatment—no matter what drug was used—especially over a short period, were almost invariably good. Now, the tuberculoid lesion is a discrete lesion which lends itself to photography and measurement. The incidence of tuberculoid leprosy in northern India according to LOWE's (1937b) latest figures is about 40 per cent. of the total cases. When it is realised that tuberculoid leprosy is easily influenced—although not necessarily cured—by various medicaments and further that it is subject to various changes

*I am indebted to the staff and patients of the Federal Settlement, Sungei Buloh, F.M.S., for much help and co-operation. I am deeply indebted to the staff of the Institute of Medical Research, Kuala Lumpur, and especially to Dr. R. LEWTHWAITE and Dr. SAVOOR who have very kindly cut and prepared some hundreds of sections of leprous material for me. To Prof. W. A. YOUNG, of Singapore, I am indebted for some helpful criticism and to the Director of Medical Services, Straits Settlements, for permission to publish this paper.

in appearance even in the absence of treatment, then we begin to understand why workers in different countries should report such conflicting results. I think it is a good thing that the attention of leprologists has been diverted for the moment from the treatment of leprosy to a study of the evolution and significance of the tuberculoid lesion.

Historical.

From the histological point of view all the main facts in regard to tuberculoid leprosy have been known for many years. Wade (1934a) gives an historical review of the subject and says Jadasshon first mentioned giant cells in leprosy in 1898. Pautrier and Boez, describing a case of tuberculoid leprosy at the Strasbourg conference in 1923, stated that Ramon y Cajal, Thoma, Mitsuda and others had previously recognized the existence of giant cells in leprosy. Hansen and Looft (1895), however, state: "in the many . . . thousands of preparations of leprous affections, which we have had under the microscope, we have never seen either a typical giant cell with marginal nuclei or caseous degeneration. There are indeed multinuclear cells . . . but never giant cells like those of tubercle." It is strange that Rogers and Muir (1925) do not mention it in their *Leprosy* although many of the cases at their disposal must have been tuberculoid. For all that, one can recognize the lesion in their illustrations. Henderson described the annular lesion and the uniformly raised plaque in 1928. The most important step in the study of the condition was made in 1934, when Wade crystallized his experiences into a systematic description of the lesion.

Clinical Features.

It would be difficult to improve on Wade's (1934a) excellent description of the tuberculoid lesion, to which the reader is referred. His illustrations are good and they make the recognition of typical lesions a comparatively simple affair. There are, however, so many divergencies from the typical, especially in that borderland where the lesion tends to degenerate to the cutaneous type, that this aspect of tuberculoid leprosy will be a fascinating and, I think, a profitable study for years to come.

For purposes of description the basic skin lesions fall under two headings: (1) the annular or "zone" lesion and (2) the raised plaque. There is a tendency for these two types to follow different courses, especially during reaction, but it would be unwise in our present state of knowledge to lay too much emphasis on minor differences. The raised plaque can, in my opinion, merge into the annular type and both types of lesion can occur at the same time in the same patient.

(*a*) The annular lesion consists typically of a depigmented patch surrounded by a narrow, raised, erythematous border. The centre is usually anaesthetic

to light touch but not completely analgesic. As a matter of interest it might be noted that it reacts to leprolin just as the surrounding skin does and may give Lewis's triple reaction on heavy stroking. The margin is raised, sometimes very slightly indeed, and often scaly. The colour of the margin in a light-skinned race—Chinese, for example—is red or dusky and is always in contrast with the depigmented centre. The margin is usually one-eighth to half an inch wide (see Figs. 1 and 2). Two or more patches may coalesce to give a serpiginous outline. The lesions vary greatly in size and number. One sees small lesions —not more than 1 inch in diameter—or again, large patches covering many square feet on the trunk.

(*b*) The raised plaque is not so easy to recognize as the annular type. Typically it is slightly raised above the level of the surrounding skin. The surface is scaly and often puckered, or it may be granular, or even warty. It has a peculiar and almost distinctive colour due no doubt to a certain amount of venous stasis in the underlying granuloma. The edge is sloping, but it may be abrupt, or again, abrupt in one direction and shelving off imperceptibly into healthy skin in another direction. There are great variations in number and in size. In some cases the lesions are small and multiple ; in other cases there are only two or three large patches (Figs. 2, 4, 5). The larger plaques are commonly met with on the face, back and about the large joints—especially near the shoulders and knees.

Pathology.

For purposes of description the pathology of the plaque is identical with that of the margin of the annular type.

The essential feature of this lesion is a dense cellular infiltration of the corium which tends to destroy the specialized structures contained therein, including the hair follicles (Figs. 9 and 10), sebaceous glands and superficial nerves. The infiltration is circumscribed, but it may extend upwards to flatten the papillae and downwards into the subcutis. Muir and Chatterji (1933) noted that it followed the course of the superficial nerves for some distance. Wade (1936), in a recent classification, reserves the term " major tuberculoid " for these extensive infiltrations.

The predominant and characteristic cell in this infiltration is the epithelioid cell, easily recognized by its oval, vesicular nucleus. The origin of this cell, which is an essential constituent of all the granulomata, has given rise to much discussion in the past. Most modern authorities agree in classifying it with the histiocytes as part of the reticulo-endothelial system of Aschoff. A more important matter is the question of the identity of this cell with the " foam " cell (lepra cell, Virchow cell) of cutaneous leprosy. Henderson (1930), in a review of the pathology of leprosy, considered that the foam cell was an epithelioid cell in which the nucleus was pushed aside by a felted mass of bacilli. Muir (1934a) and Muende in Silcock's (1934) case seem to regard

the two cells as identical. WADE (1936), however, would draw a sharp distinction between the two cells. MITSUDA (1935) found globi and LOWE (1936) found bacilli in giant cells. As the latter are presumably derived from the epithelioid cells, it is probably safe and it is certainly convenient to regard the foam cell and the epithelioid cell as identical in origin and purpose.

The infiltration in the tuberculoid lesion takes the form of more or less discrete aggregates of epithelioid cells with a sprinkling of round cells and fibroblasts especially at the periphery of each bundle. Giant cells occur frequently and even when they are not present the cells tend to be arranged in whorls as if preparatory to giant cell formation. The giant cells are of the Langhans type with a homogenous centre and radially arranged nuclei at the periphery and very like those seen in tuberculosis. There is not usually so much round-celled infiltration at the periphery, however, as there is in tuberculosis. Necrosis is unusual, but it does occur. This is the basis of nerve abscess, which must be regarded as part of tuberculoid leprosy. These abscesses are rare outside India and are probably an index of high incidence of tuberculoid leprosy.

Bacilli are difficult to find in the slowly progressive lesion, but, as will be shown later, they can be very numerous during reaction.

The above accounts—necessarily brief and by no means complete—are intended to describe lesions in which activity is at a low ebb. Leprosy is confusing, because, during the progress of the disease, the pathology may undergo a radical change. It is only by frequent biopsy that we can study the dynamic pathology of the disease. The ideal to be aimed at is continuous study of series of cases over a long period. This was not possible during my stay at Sungei Buloh, but by studying a series of cases at different stages of the condition I have tried to construct a composite picture of the progress of the disease. Illustrative cases will be quoted at this stage.

GROUP I.

TYPICAL CASES OF TUBERCULOID LESIONS IN DIFFERENT RACES.

Case 1.

No. 4,400. Chinese, male, aged 55 years. Duration of leprosy 5 years. He began as a neural leper and had a perforating ulcer of the right foot for which his leg was amputated 4 years ago. His main lesions at the moment consist of two large patches on the abdomen and flanks each covering more than 1 square foot in area. The lesions have raised erythematous margins and hypopigmented centres (Fig. 1). Anaesthesia is not complete in the centres.

*Laboratory findings.**

Nose.	Ears	Patches
R. — ve. L. — ve.	R. few. L. few.	Neck: few.

W.R. and Kahn test negative. S.I. 20. A section from one of the abdominal patches showed compact granulomatous infiltration with many giant cells. No acid-fast bacilli were seen in the section.

*W.R. = Wassermann reaction. Bacteriological findings are given as: — ve (negative), few, + ve (positive), + +, according to bacillary content. S.I. = Sedimentation index.



FIG. 1.



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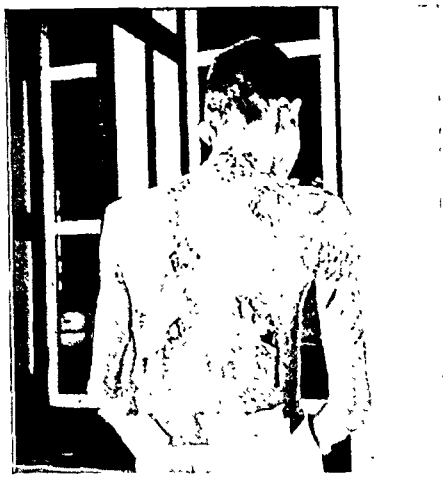


FIG. 3.



FIG. 4.

FIG. 1.—Large annular lesions on the abdomen, edges infiltrated and raised. Small patches on right cheek and on neck.

FIG. 2.—Annular and plaque lesions.

FIG. 3.—Annular lesion in reaction. Note extensive ulceration, which in some instances has spread to involve the depigmented centre.

FIG. 4.—The facial plaque in reaction. The appearance of the ear, which looks "cutaneous," is deceptive in such cases. The eye is completely closed. Paralysis of the orbicularis is an inevitable sequel.

To face page 386

the two cells as identical. WADE (1936), however, would draw a sharp distinction between the two cells. MITSUDA (1935) found globi and LOWE (1936) found bacilli in giant cells. As the latter are presumably derived from the epithelioid cells, it is probably safe and it is certainly convenient to regard the foam cell and the epithelioid cell as identical in origin and purpose.

The infiltration in the tuberculoid lesion takes the form of more or less discrete aggregates of epithelioid cells with a sprinkling of round cells and fibroblasts especially at the periphery of each bundle. Giant cells occur frequently and even when they are not present the cells tend to be arranged in whorls as if preparatory to giant cell formation. The giant cells are of the Langhans type with a homogenous centre and radially arranged nuclei at the periphery and very like those seen in tuberculosis. There is not usually so much round-celled infiltration at the periphery, however, as there is in tuberculosis. Necrosis is unusual, but it does occur. This is the basis of nerve abscess, which must be regarded as part of tuberculoid leprosy. These abscesses are rare outside India and are probably an index of high incidence of tuberculoid leprosy.

Bacilli are difficult to find in the slowly progressive lesion, but, as will be shown later, they can be very numerous during reaction.

The above accounts—necessarily brief and by no means complete—are intended to describe lesions in which activity is at a low ebb. Leprosy is confusing, because, during the progress of the disease, the pathology may undergo a radical change. It is only by frequent biopsy that we can study the dynamic pathology of the disease. The ideal to be aimed at is continuous study of series of cases over a long period. This was not possible during my stay at Sungei Buloh, but by studying a series of cases at different stages of the condition I have tried to construct a composite picture of the progress of the disease. Illustrative cases will be quoted at this stage.

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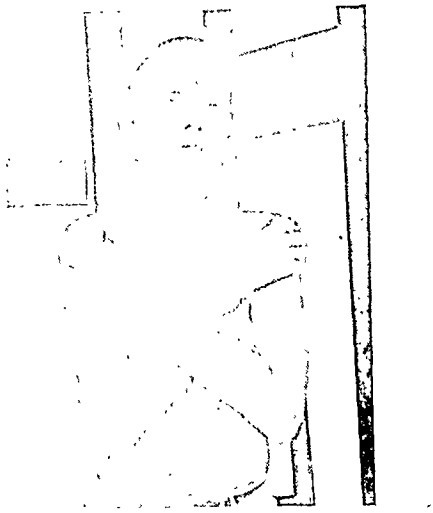


FIG. 5.

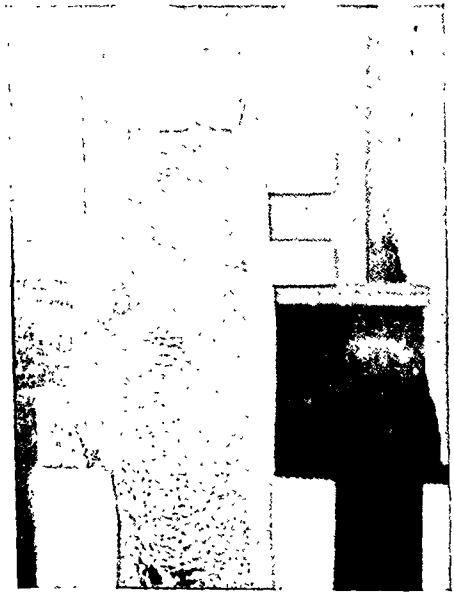


FIG. 5*a*.

FIGS. 5 and 5*a*.—In Fig. 5 paralysis of the superficial facial muscles has occurred. Note the quiescent plaques on the elbows and knee (Fig. 5*a*).



FIG. 6a.



FIG. 6.



FIG. 6b.

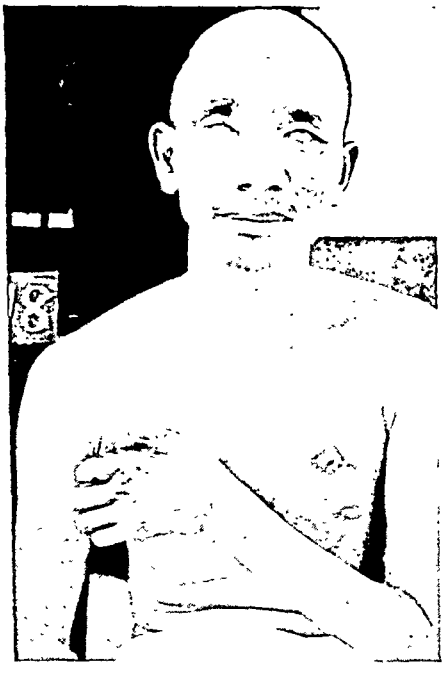


FIG. 7.

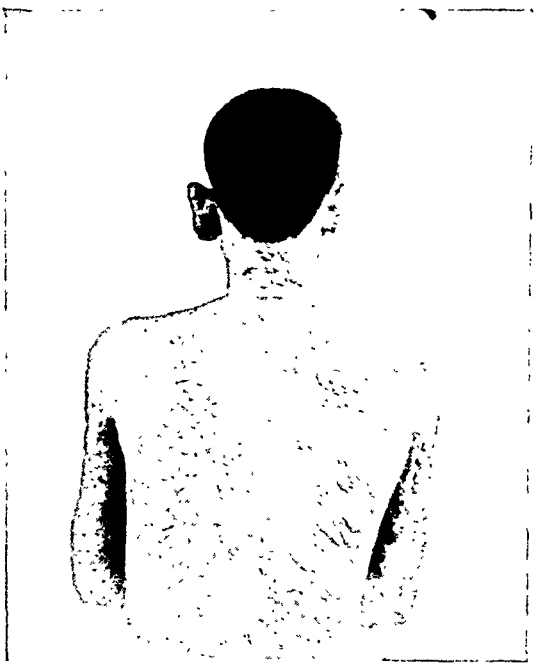


FIG. 8.

FIGS. 6, 6*a* and 6*b*.—Note again the discrete lesion with the sharply defined margin. The swelling of the lip gives a good idea of the depth of the infiltration in such a case.

FIG. 7.—The photograph shows the facial lesion and the paralysis of the orbicularis palpebrarum. The patient is trying to close his eyes. The lesion on the hand had receded considerably when the picture was taken.

FIG. 8.—Note that although the lesions are multiple, each is discrete.

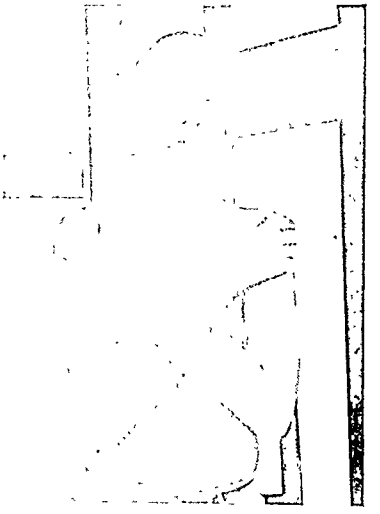


FIG. 5.



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FIG. 6a.



FIG. 6.



FIG. 6b.

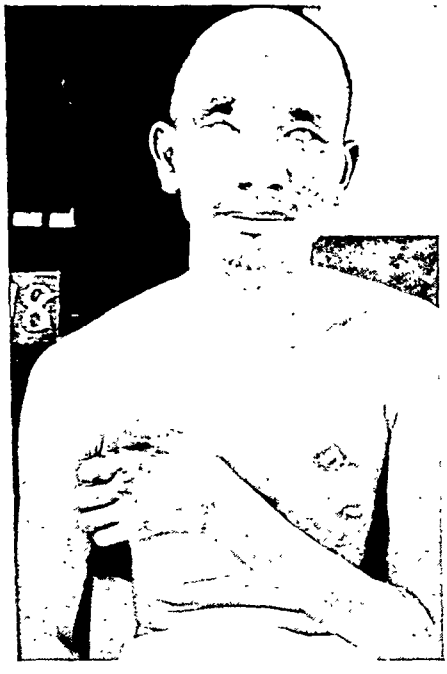


FIG. 7.

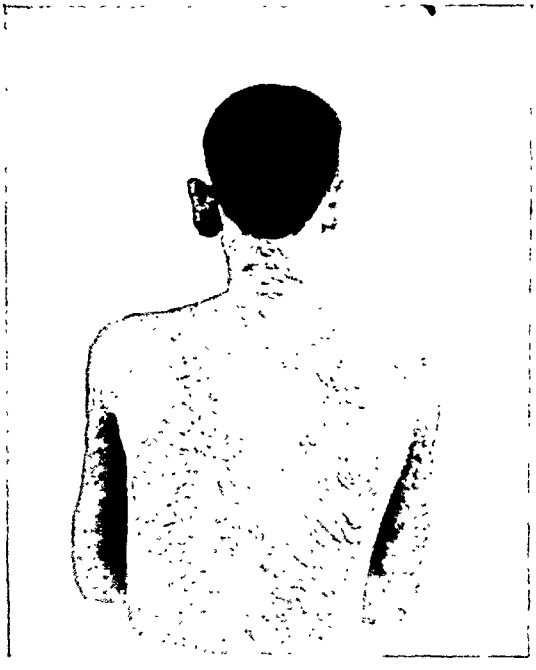


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to WADE (1934a), necrosis is rare in the tuberculoid of leprosy, though common enough in tuberculosis. The growth of the tuberculoid causes pressure on the epidermis, finally leading to erosion. This, and not necrosis, is the basis of ulceration in these cases. The growth and consequent erosion seem to be uniform over wide areas for we have often observed extensive ulceration to occur over-night.

This local upheaval must lead to the extrusion of a large number of bacilli and, apart from the public health point of view, must be regarded as a beneficial reaction. No doubt it was a reaction of this kind, ROGERS and MUIR (1925) had in mind when they discussed " elimination phenomena " in their " third stage " of leprosy.

GROUP III.

THE PLAQUE IN REACTION.

Case 4.

No. 4,569. Chinese, male, aged 36. Duration of leprosy 4 months. The disease began with infiltration of the right cheek and ear lobe. Then another patch appeared on the abdomen. The infiltration on the cheek spread to involve most of the right side of the face. Then it became uniformly raised, cyanosed and succulent, involving the ear and completely closing the right eye (Fig. 4). The abdominal patch is depigmented in the centre with a raised erythematous margin—annular type. A section from here showed typical epithelioid infiltration with a few giant cells. Bacilli were present but very scarce.

Laboratory findings :—

Nose	Ears	Patch
R. few. L. few.	R. — ve. L. — ve.	Neck : — ve.

W.R. and Kahn tests negative. S.I. 18.

This case is interesting because it shows a typical reaction in a plaque, while the annular lesion is quiescent.

The infiltration in such cases goes very deep into the tissues. The superficial facial muscles and the superficial branches of the fifth and seventh nerves get caught in the infiltration and are destroyed. This gives rise to a local paralysis and anaesthesia involving the peripheral branches of two different cranial nerves in the same situation—the " extremely peripheral neuritis " of MONRAD-KROHN (1924). The end results of this type of reaction will be seen in the next case.

Case 5.

No. 4,410. Tamil, male, aged 35 years. Leprosy began 5 years ago with ulceration of the right foot. Later fingers became mutilated. He has involvement of face, elbows and left knee. Six months ago face reacted involving eyes. There was a uniform swelling which has now receded causing paralysis of the superficial muscles of the face and ectropion of the right eye (Fig. 5).

Laboratory findings :—

Nose	Ears	Patches
R. — ve. L. — ve.	R. — ve. L. — ve.	L. arm : few.

A section from the left knee showed compact granulomatous foci with numerous giant cells. No bacilli were seen.

This case is similar to Case 4. It illustrates the end result of this type of reaction. Smears from the conjunctiva were positive for lepra bacilli. It looks as if ultimate blindness were inevitable. The lesion on the left knee (Fig. 5a) is a typical plaque tuberculoid in a Tamil. It shows no sign of reaction.

Case 6.

No. 4,532. Tamil, female, aged 18 years. Duration of leprosy doubtful. Right small toe lost 8 months before admission. First confinement 6 months before admission. Admitted to Sungei Buloh, 2.4.36. Her skin lesions appeared 2 months after her first confinement.

On admission, she had a large annular lesion extending over buttocks and thighs. There were two plaques on left arm. The left side of the face was greatly swollen and erythematous. The infiltration involved the forehead and left ear lobe. It looked not unlike a cellulitis, but the margin was discrete and constitutional symptoms were very mild. The eye was completely closed. This lesion scaled freely and went down in 3 weeks. It reacted again and she was admitted to hospital on 25.5.36, with reaction in the face and arm lesions. When the reaction subsided in the face a narrow depigmented band appeared on the border of the lesion. (See Figs. 6, 6a and 6b for varying appearances during reaction).

Laboratory findings :—

Nose		Ears	
R. few.	L. few.	R. + ve.	L. + ve.

Of four " snips " taken from different parts of the arm lesion two were negative, one showed one acid-fast and one showed two acid-fasts.

The three cases in this group call for special comment. In the first place this form of facial lepride in reaction constitutes one of the most striking features in leprosy. Once recognized they present little difficulty in diagnosis. In appearance, they are sharply demarcated, turgid, smooth and cyanosed. They have a tendency—noticeable in Case 6—to react and subside with considerable frequency. We might summarize the special features of this group as follows :—

(1) If plaques and annular lesions are present, reaction, if it occurs, will affect the plaques.

(2) The facial plaque will react before lesions in other situations whether the latter are plaques (Case 5) or annular lesions (Cases 4 and 6).

(3) Reaction in facial plaques often subsides quickly and recurs frequently.

(4) The extremely local nature of the reaction is further borne out by the absence of malaise, pyrexia, or any symptoms of general systemic disturbance.

Group IV.

Recession of lesions.

(*a*) In severe complicating disease. (*b*) Without apparent cause.

Case 7.

No. 4,018. Chinese, male, aged 45 years. Duration of leprosy 5 years. Original lesion on nose (?) annular type. Paralysis of fingers of left hand developed later and this was followed by contractures. The facial lesion had spread to involve both cheeks and lower eyelids on admission to the Settlement in June, 1935. He had intradermal and intramuscular esters and fluorescein and resorcin intravenously, by way of treatment. In January,

1936, he was admitted to the Settlement hospital with signs of active pulmonary tuberculosis. X-ray revealed infiltration of all zones of the left lung and suspicious shadows at the right base. On admission the skin lesions had all gone down. He improved considerably with rest and put on 18 lb. in weight before he was discharged from hospital in June, 1936.

As soon as his lung condition began to improve the skin lesions showed signs of activity. In the face reaction took the form of a warty hyperkeratosis with some underlying infiltration. An old depigmented lesion on the dorsum of the left hand came up as a swollen, cyanotic, glistening, discrete patch. (See Fig. 7.) A section from the hand revealed epithelioid infiltration, not as discrete as usual, and acid-fast bacilli were present. No giant cells were found.

This reminds one of a case described by MUIR (1934b). In MUIR's case the initial lesion was a macule on the face. This was stationary for some years until the patient developed typhoid. The lesion disappeared during the course of the typhoid fever, only to recur during convalescence. Later he had an attack of dysentery. Once again his leprosy disappeared and the patient thought himself cured. During convalescence, however, the leprosy recurred in a more widespread form.

In our case we see much the same train of events—recession of lesions during the course of severe disease with recurrence during convalescence. This recession of leprotic lesions in debilitating circumstances is common to all forms of leprosy. In the case of tuberculoid leprosy with lesions of low bacillary content such recession can be—and often has been—mistaken for cure. The next case to be quoted illustrates the problem in prognosis which arises in circumstances of this nature.

Case 8.

No. 3,156. Chinese, male, aged 46. Duration of leprosy 3 years. According to his account his original lesions were raised erythematous plaques on the face. He was first admitted into the Settlement on 18.2.33 and discharged on parole on 2.8.35 at which time his lesions had all subsided. He was re-admitted on 23.2.36. He had multiple lesionsall over the face, chest and abdomen (Fig. 8). The lesions were in various stages of reaction. They were raised, erythematous and discrete. The fresh ones looked red and succulent, the older ones were scaling. In the course of 2 to 3 months the lesions subsided leaving very dark patches, some of which were actually below the level of the surrounding skin. There was very little evidence of systemic disturbance in the way of pyrexia or malaise during the period of reaction.

Laboratory findings :—

Nose		Ears		Patch
R. — ve.	L. — ve.	R. ++.	L. ++.	Abdomen ++

W.R. and Kahn tests negative. S.I. 18. A section from one of the patches on the trunk showed dense granulomatous infiltration with very few giant cells. Acid-fast bacilli were present in large numbers.

Here is an important case from many points of view. In the first place, he was discharged on parole 6 months before re-admission. At the time of discharge we can take it that he was clinically and bacteriologically free from signs of leprosy. And yet 6 months later he had widely disseminated active lesions. On enquiry it was found that he was treated by intramuscular injections of hydnocarpus esters during his first sojourn in the Settlement.

In the second place the high bacillary content of his lesions should be noted. At the same time the histological findings were those of tuberculoid leprosy. Giant cells were actually present although very few in number. It was interesting to reconcile the presence of numerous bacilli with giant cells in the section. It will be noted that Case 3 presented similar findings.

Effects of Treatment.

It is not proposed to discuss fully the influence of treatment on the course of tuberculoid leprosy. Of the cases in Group III—reacting plaques—two came into the Settlement in a state of reaction. One case in this group (*Case* 4) was given tuberculin, a second had no treatment. The third had hydnocarpus treatment before reaction. *Case* 7 had a variety of treatments including hydnocarpus and various organic chemicals. *Case* 8 relapsed after becoming " clinically cured " on orthodox hydnocarpus treatment. Wade (1934c) quotes a case of reaction in a tuberculoid leper brought on by excess potassium iodide. A cursory glance through the literature is sufficient to emphasize the fact stated at the beginning of this article, viz., that therapeutists in leprosy have unwittingly concentrated their best efforts on the tuberculoid lesion. Muir (1934b) has already emphasized the fallacy of taking recession of lesions as a criterion of cure. To appreciate this it is necessary to bear in mind the initial paucity of bacilli in these tuberculoid lesions. A debilitating disease or the indiscriminate injection of toxic chemicals can and, in my experience does, cause the lesions to disappear temporarily. On recurrence, the cutaneous rather than the tuberculoid response is just as likely to be seen. Meanwhile, during the period of recession, especially in the beginning, the case may quite easily be bacteriologically negative and this, with the clinical silence, has been responsible for a host of spurious cures. A " temporary benefit in a certain number of cases " is a phrase all too familiar to the student of leprosy. The position is that we have only begun to recognize the peculiarities of the tuberculoid lesion. When we know more about its natural history then we can begin experiments designed to assess the influence of treatment.

Relative Incidence of Tuberculoid Leprosy in the Different Races in Malaya.

A survey of the inmates of Sungei Buloh Settlement was carried out by the writer in 1936. It was not found possible to examine all the Chinese and Indian adults, but all the children and all the Malay adults were examined. For various reasons which it is not necessary to detail here it can be taken that the group of adult Chinese and Indians examined constituted a random sample of their respective races in the Settlement. To make the survey complete all doubtful

cases should have been submitted to biopsy or prolonged clinical study. The population should have been grouped by duration of disease as well as by age, sex and nationality. Such a laborious procedure was out of the question but a certain amount of useful information can be gleaned from the comparatively superficial study which was carried out. The results of the survey are set out below.

TABLE I.

INCIDENCE IN ADULTS OF THE VARIOUS RACES.

Number Examined.	Nationality.	Sex.	Tuberculoid.	Doubtful.
581	Chinese	M	66 (11·3%)	38 (6·3%)
201	Chinese	F	9 (4·5%)	11 (5·5%)
166	Tamil	M	17 (10·2%)	15 (9%)
23	Tamil	F	3 (13%)	1 (4·3%)
50	Malay	M	2 (4%)	nil
26	Malay	F	nil	nil

TABLE II.

INCIDENCE IN CHILDREN AND YOUNG ADULTS BY NATIONALITY AND SEX.

Number Examined.	Nationality.	Sex.	Tuberculoid.	Doubtful.
2	Tamil	M	1	nil
4	Malay	M	nil	nil
40	Chinese	M	1	1
2	Tamil	F	nil	nil
2	Malay	F	1	nil
29	Chinese	F	1	1
Total children examined 79			Tuberculoid 4 (5%)	

The diagram shows the results at a glance. Only those who were definitely tuberculoid are included.

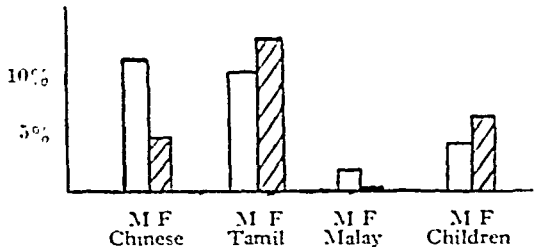


Diagram to illustrate the relative incidence in the different races and in children (7–18 years). M=Male ; F=Female.

Before proceeding to the interpretation of the figures revealed by the survey there are a few items which call for comment.

Under the heading " Tuberculoid " I have put only those cases which had typical discrete lesions and which would certainly show a tuberculoid picture on biopsy. The " Doubtful " cases are a miscellaneous group. There were lesions of the plaque type which might have been cutaneous on the one hand, or neural with a slight hyperkeratosis on the other hand. There were annular lesions with or without depigmented centres and in which the margins were erythematous and soft without being scaly. There were depigmented macules especially in Tamils, in which an erythematous border was not very definite. Finally, there were cases similar to *Case* 5 who had parchment-like lesions probably denoting the existence of old reacting plaques.

It is hardly necessary to point out that these figures relate to a community of segregated, more or less advanced, lepers. The incidence of tuberculoid leprosy here is bound to be lower than in the community outside, although the relative incidence in the different races will probably be about the same. For obvious reasons the early tuberculoid will tend to conceal his lesions unless they are in an exposed situation, e.g., the face. By the time they are segregated no doubt many will have become cutaneous.

The survey reveals a low incidence in Malays. The incidence in Chinese is surprisingly high, equalling that in southern Indians. Generally speaking, Tamils show a high incidence of neural leprosy, while the cutaneous type is more common in the Chinese. LOWE (1936) states that he did not find the tuberculoid lesion common in Dichpali—I take it he was working with Tamils and Telegus. There is, therefore, an enormous difference between the figures for the southern and for the northern Indian. This fact should be of interest to the student of the epidemiology of leprosy.

The incidence in children—all nationalities—is low. The interpretation of this fact presents some difficulties at first sight. MUIR, at various times, has insisted that the cutaneous lesion is the most likely manifestation of leprosy to occur in children. On the other hand, I have noticed at the skin clinic at the General Hospital, Singapore, that a very high proportion of the leprosy cases which come up for diagnosis are tuberculoid. Many observers will, I believe,

agree with this finding, viz., that the tuberculoid lesion is more commonly found in the early stages of leprosy. A study of the age at which the signs of leprosy first appear throws some light on the matter. Among others, MUIR and ROGERS (1925) and, more recently, LOWE (1937a) have produced graphs to show the age at which the signs of the disease are first manifest. The peak of such a curve is always in the second or third decades. Hence the early case is not necessarily that seen in a child. Actually some of the earliest lesions are seen in adult life and middle age and some of the most advanced cutaneous cases in Sungei Buloh are in Chinese under 18 years of age.

Figures for the world distribution of tuberculoid leprosy are not yet complete. It must be remembered, after all, that the recognition and study of the lesion are comparatively recent events. One gathers, however, from the available literature that the highest incidence is in northern India. The incidence among Chinese in Sungei Buloh is unexpectedly high. It should show a closer parallelism with the incidence of the neural type if the tuberculoid lesion is to be regarded as a sub-type of neural leprosy. The incidence among Malays is seen to be low and, I gather, this also applies to the Filipinos—a closely related race—and to the Japanese. MOISER (1935) gives the impression that the disease is not common in some parts of Africa. Perhaps future work may alter this impression. At the moment, the indications are that the incidence is high among these races that have been affected from time immemorial, e.g., the northern Indians, comparatively common among the Chinese and much rarer among the more recently infected Malays and Filipinos.

THE SIGNIFICANCE OF TUBERCULOID LEPROSY.

What does tuberculoid leprosy mean to us? How does the recently accumulated knowledge of the subject affect current ideas on leprosy as a whole? WADE, who has done so much valuable work on the condition, has always regarded it as a sub-type of neural leprosy. In his more recent work (WADE, 1936), I consider that he rather minimises the importance of the tuberculoid lesion. He somehow gives the impression that it can never transcend in importance and significance the parent neural leprosy. No doubt from the clinical, and perhaps from the pathological, standpoint such a view is justified but there are many other considerations which demand a broader outlook on the whole subject.

In tuberculosis we are familiar with the following types of tissue response to tuberculin or to the living bacilli :—

1. No reaction to tuberculin in the non-immune untainted child.
2. Ghon's focus in the lung—a response which involves the regional glands.
3. Chronic pulmonary tuberculosis—a local epithelioid-giant-cell response. In rapidly advancing disease, especially in primitive races, giant cells are often absent. I gather from our local pathologists that this is often the case in Malays, in whom the disease is apt to run a rapid course.

4. In pregnancy and severe disease, including the later stages of pulmonary tuberculosis, reaction to tuberculin does not occur.

5. We might regard epituberculosis as the " acute reaction " of chronic pulmonary tuberculosis.

In leprosy we recognize many types of response in the skin, nerves and internal organs which are difficult to classify. MUIR (1934a) considered that the cellular exudate in the affected nerves was always less than that in the skin in individual cases. He excised pieces of skin with thickened nerves attached and so was in a just position to compare data. Sections of the internal organs are from postmortem specimens and are not strictly comparable with biopsy material from the skin during life. It is a common experience in leprosy to find the internal organs flooded with bacilli without any sign of recent exudate in cases of death from wasting diseases, e.g., cancer, tuberculosis, etc. This, however, should not be interpreted as a manifestation of complete indifference to the presence of the bacilli, but rather as a depression of reaction in the terminal stages of the intercurrent disease. Indeed, I have good reason to believe from a study of sections that there is a cellular response in the kidneys and liver in many cases of leprosy, leading ultimately to chronic focal nephritis and to hepatic cirrhosis respectively.

HENDERSON (1930) has described three types of cellular response in the skin, putting them in chronological order.

1. The chronic inflammatory type of exudate consisting of " small round cells " with a few epithelioid cells and occasional fibroblasts. Bacilli are scarce.

2. The tuberculoid lesion, characterized by an exudate of epithelioid cells with or without giant cells. Bacilli may be very difficult to find in quiescent lesions.

3. The cutaneous type of exudate in which the " lepra cell " predominates. Bacilli are present in enormous numbers.

We might put forward a tentative classification of the varied responses to lepra bacilli and to leprolin on the following lines.

1. Indifference to leprolin in the untainted child and tolerance (*a*) in debilitating disease and (*b*) in advanced cutaneous leprosy.

2. The simple chronic inflammatory type of exudate—chiefly " small round cells " with few bacilli. The injection of leprolin produces a local reaction.

3. The epithelioid-giant-celled (tuberculoid) response. Bacilli are scarce. Acute reaction—a purely local phenomenon—is an intensification of the process and is often associated with increased bacillary content. Leprolin produces intense reaction and may, according to MUIR (1934a), lead to giant cell formation, caseation, etc.

4. The cutaneous type of response. There are enormous numbers of bacilli and relatively few cells. Acute reaction—lepra fever—is an

intensification of the local response plus systemic involvement in the way of fever, etc. The injection of leprolin produces little or no reaction.

5. Tolerance to leprolin and to lepra bacilli in severe debilitating disease. A similar phenomenon is, for all practical purposes, unknown in human tuberculosis. STEINBACH (1935) records that the albino rat can be infected with human bacilli and live for many months without any signs of disease. At autopsy, although there are no lesions, even microscopically, the tissues can be shown to contain bacilli by the results of guineapig inoculation. In man there is a similar tolerance to the presence of lepra bacilli during severe disease. MUIR (1934a) states—and I can confirm this—that the lesions often recede to such an extent that previously nodular cases may present an appearance calculated to deceive even those with a considerable experience of the disease.

The local and general reactions of tuberculosis and leprosy are often explained on the basis of allergy. There is considerable justification for such a point of view, but the mechanism of the reactions is much more complicated, especially in leprosy, than in simple allergic conditions like hay fever or bronchial asthma. The latter conditions are very much all-or-none responses to specific stimuli. In tuberculosis, as we have seen, there are gradations in the intensity of the response. Even so, the intensity of the response is in great measure roughly proportional to the amount of bacilli present. In leprosy it seems as if the immunity mechanism of the body was geared to produce different types of response at different levels of bacillary content, but the response is not necessarily in direct proportion to the bacillary content. In tuberculoid leprosy we find an exudate containing an enormous number of cells per unit of bacilli. If an advanced cutaneous case responded in a similar manner the resulting tuberculoma would fill St. Paul's.

Probably the first response to the presence of the lepra bacillus is a perivascular infiltration of " small round cells " as described above. This—the initial lesion—will probably be in nervous tissue. No doubt there are many cases in which this is sufficient to eradicate the infection. In the event of its being unsuccessful there are many possibilities to consider.

1. A local equilibrium may be established between the bacilli and the tissue. A similar equilibrium is thought to occur in tuberculosis. This would be just sufficient to confine the bacilli *in situ*. Organization of the exudate in the affected nerve will lead to increasing palsy and anaesthesia. The response in such cases, although teleologically sound, may lead to appalling results in the way of disease. Quite a small infection in the sciatic nerve, for example, may lead to complete disorganization of the foot.

2. It seems that tuberculoid leprosy can occur in three different sets of circumstances.

(*a*) As the equilibrium shifts in favour of the tissue the sensitivity to the presence of the bacilli seems to increase. This has been noted in cutaneous cases on the way to cure.

(*b*) As the infection passes from the nerves to the more sensitive skin.

(*c*) Possibly as the result of a massive dose of infection in a previously sensitized (abortive) case.

The tuberculoid response seems to be very successful in limiting the spread of the bacilli in neural leprosy. ROGERS and MUIR (1925), WADE (1934b), LOWE (1934) have all, in dealing with nerve abscess, drawn special attention to this fact. In the skin the tuberculoid response may be similarly successful. In both cases, however, we see an excessive response to the number of bacilli present.

3. The tuberculoid exudate may completely disappear and be replaced by the cutaneous type of response. The change over is in many cases accomplished after a latent period in which there is no exudate. There may be distinct improvement in neural and skin lesions as a result. No doubt the original round-celled lesion mentioned above (see 1) can degenerate directly into the cutaneous type without an intermediate tuberculoid phase. There is good reason to believe, however, that the intermediate tuberculoid phase more often occurs.

The increase in the red cell sedimentation rate, the fact that the Wassermann and similar reactions become positive, should be interpreted as evidence of a humoral or systemic response as well as a local response. There is depression of the local response as evidenced by the negative leprolin reaction, the fact that bacilli can frequently be recovered from the blood stream and the temporary amelioration of neural symptoms when the change over to the cutaneous type occurs.

Beyond a certain concentration of bacilli the tuberculoid response cannot very well occur. As the ratio between the cells and bacilli is many hundred times greater in tuberculoid than in cutaneous leprosy, it would be natural to expect a quantum, rather than a gradual, change over in some cases. Actually this does occur as evidenced by a distinct time lag in the production of lesions. During this " temporary cure " sensitivity has fallen well below the tuberculoid phase, but the bacilli may take weeks or even months to reach a concentration sufficient to evoke the cutaneous response.

4. In advanced cutaneous leprosy we often find bacilli in snips from patches of skin which look perfectly normal. Similarly, in severe disease, not alone is there no response to fresh showers of bacilli, but pre-existing lesions become flattened out. This completes the cycle ; having started at a point where there is no reaction to leprolin, we end up at a stage where there is no response to the living bacilli.

Neither the humoral nor the phagocytic theory of immunity will solve the problem of leprosy. BESREDKA'S (1927) conception of local or tissue immunity is more helpful. Response in the nerves is said to be less intense than in the skin and response in the internal organs lags behind both. In these circumstances

nerve abscess could not occur in a cutaneous case and it certainly has not been described in races in which the cutaneous type of leprosy predominates.

Acute reaction does not represent the peak of sensitivity at the level of either the cutaneous or tuberculoid response. Rather is it a recovery phenomenon. It is the expression of an urgent attempt to establish the response on the level at which it existed prior to the advent of some debilitating factor.

The tuberculoid lesion represents the peak of local response. Taking it over a large series of cases it seems to be efficient in limiting the spread of the infection. Although it is purposeful, it is, nevertheless, a stereotyped reflex and it will sacrifice an eye or a limb or a group of unimportant sweat glands with equal facility. We can in many cases depress or abolish the reaction by " heroic " methods of treatment. No doubt there are cases, e.g., lesions about the eye, in which such a step might be justified. One sees considerable clinical and bacteriological improvement occur in cutaneous cases occasionally, and cures have been described. The trouble is that even if we are successful in establishing the change over from the tuberculoid to the cutaneous response, there is still the probability that the procedure will be reversed if the bacilli decrease and then tuberculoid lesions will recur. Until we know more of the natural history of the tuberculoid lesion, until we are in a better position to predict the outcome in an individual case, it is unwise to use heroic measures to destroy such an excellent local defence against the invading bacilli.

Tuberculoid leprosy does not receive due recognition in the present classification of leprosy. To agree with WADE in regarding it as a sub-type of neural leprosy is to miss much of its significance. There is not really a sound pathological basis for the recognition of neural leprosy itself as a separate type. The exudate in an affected nerve may be simple inflammatory, " cutaneous," tuberculoid, or completely absent. Tuberculoid leprosy, on the other hand, is a definite histological and pathological entity whether it occurs in nerve or skin, whether it is quiescent or reacting, and even though it appears to assume so many different forms to an observer like TISSEUL (1936)—" primitives, secondaires, intermédiares ; en medaillon, en aires, rose-jaunâtre, parakératosique, granitée, en plateau, rosée, rouge, brillante."

SUMMARY.

1. The unrecognized existence of the tuberculoid lesion has been a source of confusion for many years in the prognosis and treatment of the disease.

2. The incidence seems to be high in those races which have been associated with leprosy for a long time. In children the incidence is relatively low.

3. Tuberculoid leprosy is a stage in the natural evolution of immunity in the disease. It represents the peak of local response.

4. Acute reaction in tuberculoid leprosy is still a local phenomenon. This is in contrast with the position in cutaneous leprosy where there is a systemic component as well.

5. Continued study of the evolution and course of the tuberculoid lesion will, it is felt certain, provide a key to the very difficult problem of immunity in leprosy and, for that matter, in many varieties of proliferative disease.

6. A more scientific classification will correlate the clinical appearances with the types of exudate present in the underlying lesions.

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DISCUSSION.

Dr. J. M. H. MacLeod : I have been much interested in Dr. HUGHES's paper and especially in his interesting description of the pathology of tuberculoid leprosy.

(1) It seems to me that there is a close analogy between certain cutaneous lesions of tuberculosis and tuberculoid leprosy. Some years ago when I first saw sections of tuberculoid leprosy I could scarcely believe that they were leprosy as they so closly resembled sections of Boeck's sarcoid. For many

years histologists have been wondering what was the precise relation between sarcoid and lupus vulgaris and it is still undecided. The relationship between tuberculoid leprosy and cutaneous leprosy is probably somewhat similar. In both sarcoid and tuberculoid leprosy there is a formative infiltration in the corium with giant cells, epithelioid cells, and occasional plasma cells and only in rare instances have bacilli been found in either of them.

The analogy of leprosy to tuberculosis has been further strengthened recently by the observation by WADE of grouped acuminate papular lesions in leprosy. A photograph of this condition which he kindly sent me showed a type of eruption similar to lichen scrofulosorum. The latter eruption is a tuberculide and due not to bacilli *in situ* in the skin but to toxins in some tuberculous focus elsewhere. It is probable that the lichenoid lepride has a similar etiology.

(2) With regard to the treatment of leprosy by extracts of, or other preparations from, bacilli the results as far as I am aware, have been unsatisfactory. I am reminded that years ago DEYCKE in Constantinople succeeded in growing a streptothrix from a piece of tissue teeming with bacilli excised from a case of cutaneous leprosy and incubated in normal saline solution. The streptothrix was regarded as a phase in the life history of the bacillus. An ethereal extract was made of the organism to which the name " Nastin " was given. This was put on the market and extensively used and reported on favourably in certain quarters. A trial of it in this country on a number of cases in private and at the Tropical Hospital at the Albert Dock gave negative results.

Dr. A. C. Howard: I have worked among lepers in Nigeria during the last few years. My impressions were, that chiefly in the south there is a tendency for the cutaneous or C3 type to predominate, while in the north the neural or tubercular type is most common. In his lecture, Dr. MUIR mentioned that one of the commonest neural signs in India was the enlargement of the auricular nerve. I have never seen an enlarged nerve in leprosy, I have palpated them once or twice but never seen them ; and I wonder whether he would explain why in different parts of the world the predominating type of the disease varies as it does : India, for instance, being very different from Nigeria in this respect.

Maj.-Gen. Sir John Megaw: When I was house physician at the Medical College of Calcutta more than 30 years ago, I came across a large number of cases of leprosy of a very mild neural type, and having read so much about leprosy being a dreadful disease, I could hardly persuade myself that these were really cases of leprosy. The majority of the cases were of such a mild nature that I was firmly convinced that many of them never went on to the serious type.

I discussed the point with the late Sir JONATHAN HUTCHINSON, and asked him whether there was any other disease in which there occurred anaesthetic patches associated with thickening of the nerve without any tendency to progress to an advanced stage, as is expected in leprosy ? His reply was that they could not be anything else but leprosy, and that he himself had been much struck by the number of mild cases which were seen in India as compared with other countries. On the whole there is a good deal of evidence to show that leprosy in India is frequently a mild disease which may be in many cases trivial. Dr. MUIR emphasized quite rightly the importance of exercise in the treatment of leprosy. But I have seen patients in leprosy institutions being exercised in the heat of the day; they were badly nourished and it struck me that in those cases the exercising process was distinctly overdone. I think the important thing, as Dr. MUIR has emphasized, is to maintain the nutrition of the patient. There is a line of treatment which we hear of from time to time, and I am rather surprised that more people do not give it a trial. I refer to the specific treatment by the leprosy antigen. A glance at one of the sections demonstrated this evening will show that there is practically a pure culture of lepra bacilli in the skin. In Lucknow 20 years ago, I had a patient with a very pronounced type of cutaneous leprosy. He had practically leontiasis, and a section from his ear lobes revealed enormous numbers of bacilli. I cut out some of the more conspicuous nodules, sliced them up in a freezing microtome, converted them into a very fine emulsion, killed them by heat, and added a little carbolic. I started injecting very small doses of this emulsion in very much the same way as tuberculin is used in the treatment of tuberculosis, and gradually increased the dose. There was no other special treatment but at the end of 6 months the patient was to all intents and purposes cured. He came back 18 months later and nobody would have suspected he ever had leprosy. He made the very interesting proposition that, as I had found a cure for him we should become partners, that I should supply the material and he should tour the country and that we should go fifty-fifty in the profits from this new treatment. Unfortunately I had not much opportunity of pursuing that method of treatment. Of course, it had been done before, though I was not aware of this at the time, and it has been done on several occasions since. But I am rather surprised that it has not been given a more extensive trial, because it sounds perfectly reasonable. We are quite familiar with the remarkable results due to chaulmoogra oil, but it is quite possible that vaccine might be a useful auxiliary line of treatment, especially as so many patients can supply enormous numbers of leprosy bacilli in a very rich culture from their skin lesions.

Dr. A. Felix: May I carry further the interesting point raised by Sir JOHN MEGAW ? It may have escaped the attention of those present here that quite recently the transmission of human leprosy to an experimental animal

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Dr. A. C. Howard: I have worked among lepers in Nigeria during the last few years. My impressions were, that chiefly in the south there is a tendency for the cutaneous or C3 type to predominate, while in the north the neural or tubercular type is most common. In his lecture, Dr. MUIR mentioned that one of the commonest neural signs in India was the enlargement of the auricular nerve. I have never seen an enlarged nerve in leprosy, I have palpated them once or twice but never seen them; and I wonder whether he would explain why in different parts of the world the predominating type of the disease varies as it does: India, for instance, being very different from Nigeria in this respect.

Maj.-Gen. Sir John Megaw: When I was house physician at the Medical College of Calcutta more than 30 years ago, I came across a large number of cases of leprosy of a very mild neural type, and having read so much about leprosy being a dreadful disease, I could hardly persuade myself that these were really cases of leprosy. The majority of the cases were of such a mild nature that I was firmly convinced that many of them never went on to the serious type.

I discussed the point with the late Sir JONATHAN HUTCHINSON, and asked him whether there was any other disease in which there occurred anaesthetic patches associated with thickening of the nerve without any tendency to progress to an advanced stage, as is expected in leprosy ? His reply was that they could not be anything else but leprosy, and that he himself had been much struck by the number of mild cases which were seen in India as compared with other countries. On the whole there is a good deal of evidence to show that leprosy in India is frequently a mild disease which may be in many cases trivial. Dr. MUIR emphasized quite rightly the importance of exercise in the treatment of leprosy. But I have seen patients in leprosy institutions being exercised in the heat of the day; they were badly nourished and it struck me that in those cases the exercising process was distinctly overdone. I think the important thing, as Dr. MUIR has emphasized, is to maintain the nutrition of the patient. There is a line of treatment which we hear of from time to time, and I am rather surprised that more people do not give it a trial. I refer to the specific treatment by the leprosy antigen. A glance at one of the sections demonstrated this evening will show that there is practically a pure culture of lepra bacilli in the skin. In Lucknow 20 years ago, I had a patient with a very pronounced type of cutaneous leprosy. He had practically leontiasis, and a section from his ear lobes revealed enormous numbers of bacilli. I cut out some of the more conspicuous nodules, sliced them up in a freezing microtome, converted them into a very fine emulsion, killed them by heat, and added a little carbolic. I started injecting very small doses of this emulsion in very much the same way as tuberculin is used in the treatment of tuberculosis, and gradually increased the dose. There was no other special treatment but at the end of 6 months the patient was to all intents and purposes cured. He came back 18 months later and nobody would have suspected he ever had leprosy. He made the very interesting proposition that, as I had found a cure for him we should become partners, that I should supply the material and he should tour the country and that we should go fifty-fifty in the profits from this new treatment. Unfortunately I had not much opportunity of pursuing that method of treatment. Of course, it had been done before, though I was not aware of this at the time, and it has been done on several occasions since. But I am rather surprised that it has not been given a more extensive trial, because it sounds perfectly reasonable. We are quite familiar with the remarkable results due to chaulmoogra oil, but it is quite possible that vaccine might be a useful auxiliary line of treatment, especially as so many patients can supply enormous numbers of leprosy bacilli in a very rich culture from their skin lesions.

Dr. A. Felix: May I carry further the interesting point raised by Sir JOHN MEGAW ? It may have escaped the attention of those present here that quite recently the transmission of human leprosy to an experimental animal

has been described. The Syrian hamster has been suggested for this purpose by Dr. BALFOUR-JONES, of the National Institute for Medical Research, and Professor ADLER, of the Hebrew University in Jerusalem reported in a preliminary note in *The Lancet*,* 18th September, 1937, the results of a successful experiment carried out on a small number of Syrian hamsters. If these results are confirmed a basis will be provided for an adequate study of the problem of specific therapy of human leprosy.

Dr. C. C. Chesterman: I would like to ask either one of the speakers what is the relative infectivity of the tuberculoid type and the cutaneous type, because it makes a great difference from the point of view of segregation. In the north-east of the Congo the majority are of the tuberculoid type, and it does not advance quickly. The annular patches remain for many years. The natives have two distinct names, one for the tuberculoid type and another for the cutaneous type. In that same area, Dr. DUBOIS has found that 33 per cent. of the people have acid-fast bacilli in the glands, although not more than 10 per cent. show symptoms of leprosy, I would like to support what Dr. HOWARD of Nigeria says. He mentioned the rarity of anything like thickened nerves. I have rarely seen that myself, and one rarely finds any dullness in the perception of the skin inside the annular eruption.

Dr. C. J. Austin (Fiji): Dr. MUIR mentioned the massacre of lepers in China, and said that possibly this had occurred in the past in Nigeria. It was not long ago that I was informed by one of the old paramount chiefs in Fiji that in an island known as De-ni-Kia in his province, a former chief used to segregate all his advanced lepers in order that he should never be without a " bokola " or a body prepared for the oven, with which to welcome his friends or important guests ! Evidently the same policy was to some extent carried out in Fiji. I think possibly Dr. MUIR was rather hard on the efforts of some of our Governments in those districts where compulsory segregation is carried out. I admit that in India and parts of Africa, this compulsory segregation is impossible. He mentions it as an ideal but seems to regard it as nothing more. In Fiji compulsion has been in force since 1911, and while we cannot say that our number of admissions is going down to any great extent, we *are* getting a far better type of case coming along than we had in the past. The idea of compulsion does quite probably cause hiding of early cases, but we are trying to get over that by the education of Fijian and other native students. Our station in Fiji is known as the Central Leper Hospital, and is to a certain extent " central " because we take cases from other island groups, Cook Islands, Samoa, Tonga and the Gilbert Islands, and we are getting in young men from

*ADLER, S. (1937). Inoculation of human leprosy into Syrian hamster. *Lancet* **2**, 714.

each of those groups to the Central Medical School in Suva. I do not know if any of you were on the British Medical Association tour last year, and passed through Suva, but if so you saw something of this school. Quite a number of people have been there, and have been surprised at the way our students are getting a grip of medical subjects. They have a four-years' course, and, as long as they are under a certain amount of supervision, do valuable work. They can educate the people in a way that we cannot do and they get to know more easily than we can if there are lepers in the village. I think we are in that way getting to the root of the problem.

With regard to Dr. HUGHES's paper and this question of tuberculoid leprosy, I wonder whether we are strictly correct in speaking of tuberculoid leprosy; whether it is a type, or whether it is not rather a question of a tuberculoid lesion occurring in either a neural or cutaneous type? He raised a further point in my mind. He states that owing to the less response or reaction of the nervous tissues one does not get nerve abscess in a cutaneous case; but I have myself operated on two cases in which I had to avoid nodules in the skin incision, so I would say it is definitely possible to get nerve abscess in a cutaneous case. One or two pictures that he shows of tuberculoid lesions of the face seem to me to give definite signs of cutaneous leprosy of the ear, and I think it is generally admitted that tuberculoid lesions can occur concurrently with cutaneous lesions. Questions of classification arise and will have to be thrashed out again at the International Congress of Leprosy, in Cairo next year, but to try to make a new type of the tuberculoid form does not seem to me to help us to understand it. Whether it can be regarded as related to nerve leprosy, as has been the tendency, is another matter. The whole point is that leprosy is a more or less regular gradation or a series of changes, no one of which can be accurately delimited from another. It may go forward to a certain stage and then retrogress. I am not quite sure whether I got Dr. HUGHES's meaning when he spoke of the results of treatment. He appeared to suggest that in the past we have been trying to overcome tissue response whereas what we should have done was to encourage the bacillus to carry on in its own way. There is no obvious disease unless the tissue responds, but there may be no tissue response for either of two reasons (1) because the body is not capable of tissue response or (2) because the organism has been overcome and, there are, therefore, no signs of leprosy. Perhaps Dr. HUGHES would go into that aspect again.

Dr. Gushue-Taylor (Formosa, Japan): On the point raised by one of the speakers in regard to enlargement of the nerve: among Chinese lepers this is quite a common feature. You need only to turn the head, putting the sterno-mastoid on the stretch and you can frequently feel the great auricular nerve: not only can you feel it but you can actually see it standing out. The point is that the manifestations of leprosy probably vary in different countries. Take for instance the question of lesions of the scalp. I remember some years

ago Dr. WADE remarking to me that leprosy did not affect the scalp. But a short time after that I went to Japan and there Dr. MITSUDA showed me scores of his patients with marked cutaneous lesions of the scalp. One speaker spoke of the danger of over-exercising your patients, but I would say from my experience that the patients will not allow anyone to over-exercise them.

Lt.-Colonel C. H. Barber: Has Dr. HUGHES come across cases where intercurrent tuberculosis has caused recession in leprosy, and can he say whether some of those cases have been not only recessed but apparently permanently cured? It seems to me possible that there is an antagonism between tuberculosis and leprosy, similar to that between smallpox and malaria, malaria and general paralysis and some other diseases.

Dr. Hughes (in reply): The similarity between tuberculoid leprosy and sarcoid has been recognized. I recently saw a case of sarcoid in Singapore and structurally it seemed indistinguishable from tuberculoid leprosy. I understand that modern dermatologists have found adenopathy and X-ray signs of pulmonary lesions in many cases of Boeck's sarcoid and that this condition is being more and more regarded as a tuberculide. I should like very much to see some of Dr. MCLEOD'S sections some time and discuss these sarcoid and lichenoid lesions with him.

Regarding treatment there is much confusion as to the effect of various remedies. The important thing to remember is that in tuberculoid leprosy one sees an enormous number of cells per unit bacillary content, whereas in the cutaneous type the numerical relation between cells and bacilli is reversed. It is quite obvious that without cellular exudate there is no disease, leprosy, as we know it. Leprosy *is* the nodular skin plus trophic lesions. These nodular lesions consist of the underlying cellular exudate and if these cells disappear there is no clinical disease. As to treatment—as I see it, any wasting disease will cause recession of these leprotic lesions and the difficulty is to distinguish between recession of lesions and cure. This may sound purely theoretical, but Dr. MUIR has actually shown a picture in which it was difficult to decide whether the patient was a leper or not although the skin contained large numbers of bacilli. I have seen cases of cirrhosis of the liver in which you could not say whether the patient was a leper or not and yet, if you took a " snip " you would find the skin teeming with bacilli—this, too, from parts of the skin which looked perfectly normal. What would happen eventually if the bacilli continued to propagate I do not know. I have seen some cases where I got the impression that there was as much bacillary substance as there was tissue.

The effect of drugs in heroic doses seems to be identical with that of wasting disease. Lesions recede—the cellular exudate is absorbed—clinical cure is established and, unfortunately, too often recorded. In my own short experience

—twelve months all told—the impression I got was that less than 1 per cent. of cases subjected to drastic treatments were cured eventually. Clinical "cures" might amount to 50 or 60 per cent., but the permanent results were bad. Hence it is only with the greatest caution one should recommend a new treatment of leprosy. What we want really is more information about the progress of the disease and the effect of such factors as race, climate and nutrition on that progress. We want to know the natural history and evolution of every form of the disease before we can assess the influence of any particular form of treatment. In view of the fact that recession of lesions can be produced equally well by either drugs or debility, any new form of treatment must be subjected to the most searching criticism if we are to avoid causing harm to lepers by our misguided enthusiasm.

With regard to nerve abscess, it was interesting to hear Dr. AUSTIN say he met the condition in cutaneous leprosy. If Dr. MUIR's contention that reaction in nervous tissue is always less than that in the skin it seems impossible that nerve abscess should occur in cutaneous cases. The condition is rare outside India, and I have no recollection of meeting a typical case in Malaya. One would like to hear full details of Dr. AUSTIN'S cases as it seems almost impossible to visualise a pure tuberculoid response in the nerves and a frank cutaneous response in the skin, simultaneously. I am sorry that our time is limited and that it is not possible to carry the discussion on nerve abscess any further to-night.

Dr. Ernest Muir (in reply): There are one or two points to which I should like to refer. There is the question of finding nerve abscess in cutaneous leprosy; I have seen it in three or four cases. When it occurs I think the patient has probably been originally of the neural type, and has then developed caseous lesions inside his nerves. Later his health deteriorates and the disease advances to the cutaneous type. At some subsequent period during lepra reaction the caseous material liquefies, and a nerve abscess forms.

As regards the finding of nerve leprosy more commonly in some places than in others, Dr. HOWARD suggested there was less nerve leprosy in Southern Nigeria. That was the impression which I also gained during my short visit there last year. But as a matter of fact two thorough surveys have been made in limited areas in the Onitsha and Owerri Provinces during the last few months. In one of these surveys, out of 27,900 examined 2·4 per cent. were found to have leprosy, though the probable actual percentage is 3·1 per cent. But out of these, 97 per cent. were scheduled as neural. Much depends on the standard of classification; and still more depends on whether the total population is examined, or only those who attend for treatment or present themselves for admission to a leper settlement. Nerve leprosy is exceedingly common in India, and why it is not so common in Malaya I cannot say; all I can suggest is that they are not looked for by a thorough survey. I may point to the picture

I have shown tonight of a patient who had a caseous auricular nerve, with an abscess forming. As a matter of fact that patient was sent to me from Singapore. Many doctors say they have never found leprosy attack the cutaneous nerves, but when I have pointed it out to them in cases where they had previously failed to find it, they have realized that it is very much more common than they imagined. I may say it was only after some years experience that I found the nerves thickened to anything like the extent I did latterly.

The other question was the relative infectivity of tuberculoid and skin leprosy. I think that there is no doubt that the tuberculoid, if it is infectious at all, is very much less so than the skin type. I think everybody agrees on that point. There is always the danger that the tuberculoid or neural case may pass on into the infectious cutaneous type of leprosy.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

COMMUNICATIONS.

FILARIASIS OF GROUND DOVES IN ST. CROIX, VIRGIN ISLANDS.

BY

F. W. O'CONNOR, M.R.C.S.

AND

H. A. BEATTY.*

On 22nd December, 1936, while searching for an animal naturally infected with sheathed microfilariae, we shot a ground dove, *Columbigallina passerina nigrirostris* (Fig. 1). Sheathed microfilariae were abundant in the heart blood but scanty in the peripheral blood from the wing. In fresh blood mixed with heparin it was subsequently found that the microfilariae exhibited progressive

*From the Department of Medicine, Presbyterian Hospital, Columbia University, New York, and the Department of Health, St. Croix. We are grateful to Dr. JAMES KNOTT, Civil Medical Officer, St. Croix, for facilities arranged for doing this work and for making available the help of the co-author.

Owing to the death of Professor O'CONNOR, Col. CLAYTON LANE kindly undertook the responsibility of seeing this and the following paper through the press.—ED.

as well as wriggling movements. Stained specimens were, at our request, demonstrated before the Royal Society of Tropical Medicine by Col. CLAYTON LANE (O'CONNOR, 1936). In several doves examined adult parasites were found in large blood vessels beneath the liver and between the abdominal organs and elsewhere. An infected bird was sent to Dr. DONALD L. AUGUSTINE (1937) who named the parasite *Vagrifilaria columbigallinae*. Because of the similarity of both adult worms and microfilariae (Fig. 2) to the corresponding stages of *Wuchereria bancrofti* in man and because of the interesting pathology associated with the bird parasites, we decided to make further studies of the latter in the hope that some light might be thrown on the kindred human infection.

Up to date, the heart blood of sixty-five ground doves has been examined in St. Croix and in sixty-three or 96·9 per cent., the same sheathed microfilariae have been found. On the other hand, in four ground doves of the same species kindly collected and sent to us by Dr. J. P. O'MAHONEY from Antigua, serial section failed to reveal any evidence of infection. A similar microfilaria was once found in each of the following birds: the white headed pigeon, *Columba leucopala*, the red headed pigeon, *C. squamosa* and the mountain dove, *Zenaida zenaida*, but studies of more birds of these species indicate that they are not commonly infected.

Method of Study.—Having ascertained that the majority of adult parasites were located in the abdomen or chest, the internal organs from the throat to the anus were removed in one piece. This was of suitable size for embedding in one paraffin block and the resulting serial sections were found to fit conveniently on an ordinary microscope slide under a No. 1 coverslip 22 × 50 mm. Such sections revealed in their normal anatomical relationship the lungs, heart, gizzard, liver, spleen, pancreas, intestines, kidneys and sex organs.

Forty-nine doves were studied by serial section. In one no evidence of infection was found and *no pathological changes* were observed. In another while microfilariae were found in the organs and blood vessels, the adults were not discovered, indicating that the latter were in tissues outside the thorax and abdomen. In the remaining forty-seven doves recently-living adult worms were found in the following locations—in blood vessels in the neighbourhood of the liver forty-four times (Fig. 3), lungs nine times, pancreas six, and heart six; and between the coils of the intestines four times. Dead and degenerating adult worms were found in sixteen birds; eleven in the liver and five in the lungs. Degeneration was always by caseation and fragmentation, the dead worms being surrounded and invaded by foreign-body giant cells and small round cells with eosinophils. Calcification of dead worms was not observed.

In sections of the brains and eyes of four doves a few healthy microfilariae were found, but as they were not associated with any observable pathology these organs were not subsequently studied. In the very small spleen micro-



FIG. 1.—*Columbigallina passerina nigrirostris* (natural size).

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FIG. 2.—*Microfilaria bancrofti* and *columbigallinae* from fresh blood, compared. Both same magnification.
(*a*) *Mf. bancrofti.* (*b*) *Mf. columbigallinae.*



FIG. 3.—Section through liver of dove showing mass of male and female worms in dilated blood vessel. × 28.

FIG. 4

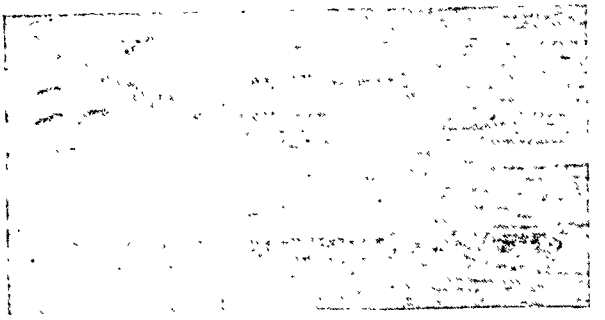


FIG. 5*a*

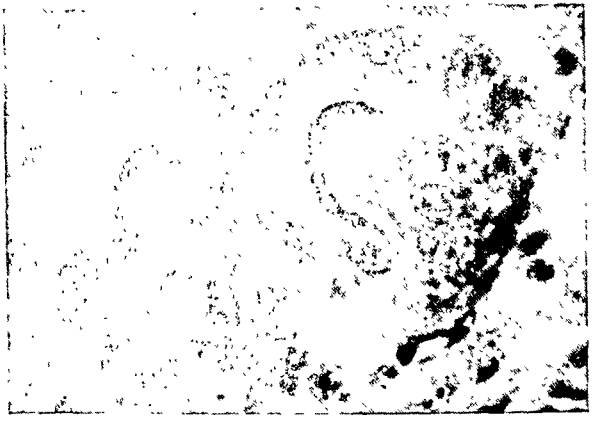


FIG. 5*b*.

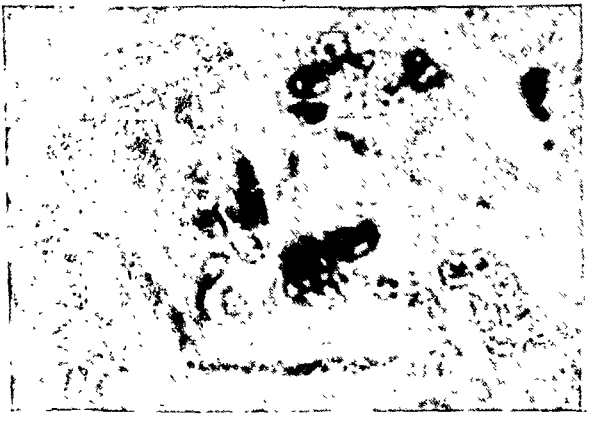


FIG. 4.—Microfilariae between muscle fibres of heart of ground dove. × 970.

FIGS. 5*a* and 5*b*.—Degenerating microfilariae in giant cells of liver of dove. × 970.

filariae were not conspicuous and no sign of degeneration was seen. In the skin, gizzard, sex organs and pancreas microfilariae were not present in large numbers. In the kidneys embryos were easily found between the tubules and sometimes in masses in the large vessels. On only a few occasions were they found in the diminutive glomeruli. In the intestines several microfilariae, sometimes in groups, were found in the submucosa especially at the bottom or in the middle of the villi. A few parasites were occasionally found to be degenerating in these situations but never with the regularity to be described elsewhere. In the *lungs* microfilariae were usually present in large numbers *at any time of day or night* even when adult worms were present only in the liver. Sometimes in a section masses of embryos were present in large and small lung vessels while close by in similar vessels they were scanty. They were also very numerous in the blood of the heart chambers. In all these situations the majority of the parasites were in mid-stream* and showed no tendency to adhere to the sides of the vessels or chambers. In the heart wall microfilariae were often very numerous and were frequently seen straightened out between the muscle fibres (Fig. 4). The embryos in lungs and heart exhibited clear morphology and appeared to be perfectly healthy, nor was any cellular response occasioned by their presence.

The most striking changes were found in the *liver* which was generally enlarged in proportion to the number of adult worms in the body and of microfilariae in the general circulation. In the livers of thirty-four doves, on holding to the light sections stained with haematoxylin and eosin, local areas of smaller or larger size could be seen which were much bluer than the rest of the hepatic tissue. These areas were frequently at or near liver margins and were sharply differentiated from the adjacent healthy tissue. Sometimes only one such area was found but at other times several islets of such tissue were observed. The position of these areas did not seem to bear any relationship to the site of adult parasites in the liver and were found in doves in which parent worms were present only in the lungs. On microscopical examination, the " blue " areas were found to be heavily infiltrated with lymphocytes amidst which were a large number of multinucleate giant cells. Varying numbers of eosinophil leucocytes were scattered or grouped in the vicinity. While microfilariae, generally dead and degenerating, were scattered throughout the liver they were much more numerous in the " blue " areas and especially in the giant cells (Figs. 5*a* and *b*). In one section, 7μ thick $\frac{1}{3}$ inch square, 336 giant cells were counted and, in 139 of these, degenerated microfilariae were found. Examination of serial sections showed microfilariae in many of the other giant

*It should be noted that the majority of the birds were killed instantly by shooting or by chloroform and that within a minute the internal organs after removal were placed in 10 per cent. formalin for fixation.

cells. Owing to the curled position of the dead embryos, the giant cells frequently appeared like a spiral nebula. In the livers of eleven doves giant cell formation was not present but islands of dense round-celled infiltration with eosinophils were conspicuous. In many of these islands degenerating microfilariae were seen. In two livers there was no such marked cellular reaction and it is worth noting that in both of these cases very few microfilariae were present in the general circulation; nevertheless, dead and degenerating microfilariae were found between some of the liver cells. In all cases where present, the dead microfilariae showed every stage of degeneration varying from cloudy specimens, with otherwise clear morphology, to forms in which a chain of fragmented nuclei alone revealed their presence. The whole picture seen again and again in the different livers suggested massive death, degeneration and absorption of parasites going on continuously in the organ.

The method of transmission of the dove filaria was not determined. Immediately after shooting birds, a number of *Olfersia americana* living on the doves were captured and dissected but no microfilariae or larvae were found in these insects. Believing that possibly the birds might be infected while still in the nest, six squabs, about 10 days old, were serially sectioned but no parasites were found. The only suggestive finding was perivascular infiltration of some of the portal vessels with eosinophil leucocytes.

Periodicity.—Evidence of periodicity of the microfilariae was not observed although two methods were adopted to ascertain if the microfilaria of *Vagrifilaria columbigallinae* exhibited any.

From the wing veins of two doves 10 c.mm. of blood was drawn into a syringe containing heparin every 2 hours for 24 hours and the microfilariae counted. It appeared at first that there was some decrease in numbers of the embryos between 10 a.m. and 4 p.m., but control specimens from an adjacent wing vein frequently showed the large numbers of parasites that had been found at night.

A batch of twelve doves were captured and, after finding that all had microfilariae in the blood of the wing vein, one was killed with chloroform every 2 hours for 24 hours. Immediately after death the internal organs were placed in 10 per cent. formalin and were subsequently examined by serial section. No evidence of periodicity resulted from the study of the sections. Most of the parent female worms contained embryos and ova in varying numbers and in the vagina microfilariae were stretched in bundles. On the other hand, there was no evidence of continuous parturition and frequently embryos were noticeably scanty near the parent worms although abundant in lungs and heart. No microfilariae were found issuing from the vagina in sections showing that area of the worm. No collapsed worms suggestive of recent parturition

were seen. Two female worms were considered immature because they were smaller than usual and because there was no evidence of either ova or microfilariae in the uteri. No collapsed parent females, like those seen in some human cases were discovered. It may be noted that in the same section of the uterus nearly fully developed microfilariae and partially developed ova were seen together.

The findings in one dove seem worth mentioning. Killed and examined at 10 p.m., very few microfilariae were found in any of the blood vessels or organs although a few degenerating ones were found in giant cells of the much enlarged liver. No living adult worm was found but a recently dead female containing ova and some microfilariae was present in the lung. The parasite was surrounded by a zone of giant cells and lymphocytes with eosinophils. The findings suggest that with the death of the parent worm not only the latter but also the liberated microfilariae degenerate rapidly. An extensive pathology of the liver was judged by Dr. ALVIN PAPPENHEIMER to be due to adeno-carcinoma involving especially the biliary ducts. The few microfilariae found outside the liver, e.g., lungs, heart, etc., were undergoing degeneration. With this exception degenerating microfilariae were only marked in the livers and occasionally in the intestine.

During examinations of the sections, the following intestinal parasites were noticed, all in the small intestine ; a tapeworm with hooks on the rostellum twice, an oviparous nematode worm four times, and coccidia twice.

SUMMARY.

1. Of the ground doves examined in St. Croix *Microfilaria columbigallinae* was found in the blood of 96·9 per cent.

2. The adult worms were most commonly found in the liver, less often in the lungs, heart, pancreas and tissues of the intestines.

3. The microfilariae were most numerous in the lung vessels and heart chambers and heart muscle ; in these situations they appeared to be healthy.

4. Massive destruction of microfilariae was constantly observed in the liver associated with varying degrees of cellular response.

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Professor O'CONNOR died 4 days after the date of the postmark on the envelope enclosing the paper. In his accompanying letter, he emphasized, with italics, that this paper was a *Prelude* to a larger one which he was beginning at once on " massive destruction of microfilariae in man : 1, in the lymphatics before reaching the blood stream (four cases), and 2, in the liver and spleen after reaching the blood stream (probably six cases)."

CLAYTON LANE.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

WUCHERERIA BANCROFTI IN MOSQUITOES OF ST. CROIX.

BY

F. W. O'CONNOR, M.R.C.S.,

AND

H. A. BEATTY.*

The present work was undertaken in the hope that better acquaintance with the fate of *Wuchereria bancrofti* in some of its arthropod hosts might reveal information indicating methods for the prevention of Bancroft's filariasis.

SCOPE OF THE PRESENT STUDIES.

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1.—GENERAL INFORMATION ABOUT ST. CROIX.

The island of St. Croix (Fig. 1) lies between 17th and 19th parallels north latitude and 64° 10′ and 65° 30′ west longitude. It comprises 84 square miles. The length is 21 miles, while the breadth, which varies considerably, is at most 6 miles. There are two large towns, 15 miles apart, Christiansted, the capital, on the north and Fredericksted, the principal port, on the west coast. The surface is of sandy loam grading off into gravel and the land is very porous, in consequence there is little surface water as compared with the neighbouring

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The laboratory facilities for these studies were made available by Dr. JAMES KNOTT, Civil Medical Officer, St. Croix, who also lent the services of the joint author. We acknowledge our cordial thanks for the assistance.

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AUGUSTINE, D. L. (1937). Description of a new filariid from ground doves of St. Croix, Virgin Islands. *Ibid.*, 31, 47.

Professor O'CONNOR died 4 days after the date of the postmark on the envelope enclosing the paper. In his accompanying letter, he emphasized, with italics, that this paper was a *Prelude* to a larger one which he was beginning at once on " massive destruction of microfilariae in man : 1, in the lymphatics before reaching the blood stream (four cases), and 2, in the liver and spleen after reaching the blood stream (probably six cases)."

CLAYTON LANE.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

WUCHERERIA BANCROFTI IN MOSQUITOES OF ST. CROIX.

BY

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AND

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The present work was undertaken in the hope that better acquaintance with the fate of *Wuchereria bancrofti* in some of its arthropod hosts might reveal information indicating methods for the prevention of Bancroft's filariasis.

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1.—GENERAL INFORMATION ABOUT ST. CROIX.

The island of St. Croix (Fig. 1) lies between 17th and 19th parallels north latitude and 64° 10′ and 65° 30′ west longitude. It comprises 84 square miles. The length is 21 miles, while the breadth, which varies considerably, is at most 6 miles. There are two large towns, 15 miles apart, Christiansted, the capital, on the north and Fredericksted, the principal port, on the west coast. The surface is of sandy loam grading off into gravel and the land is very porous, in consequence there is little surface water as compared with the neighbouring

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The laboratory facilities for these studies were made available by Dr. JAMES KNOTT, Civil Medical Officer, St. Croix, who also lent the services of the joint author. We acknowledge our cordial thanks for the assistance.

island of Puerto Rico. The average rainfall is 46 inches; the dryest months are between January and March after which the rainfall rises until it is highest in October; from July to October hurricanes are liable to occur. For the year 1st July, 1935, to 30th June, 1936, the general average maximum temperature was 89° F., the minimum 66° F.; the maximum temperatures were 90° F. in July, 92° F. in August, 91° F. in June, 64° F. in November, 61° F. in December, 62° F. in February and 60° F. in March.

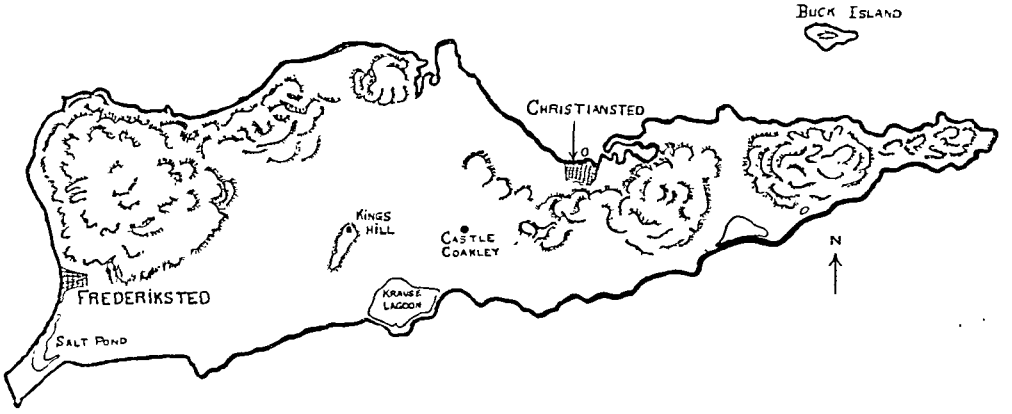


FIG. 1.—ST. CROIX, VIRGIN ISLANDS, U.S.A.

According to the latest census (1930) the population of the island was 11,413; 414 of them are white, 9,592 negro, 1,386 mixed colour, and 21 Asiatic. In the Christiansted area, with which we are most concerned, the population for the same year was: white 164, negro 3,126, mixed 475 and Asiatic 2.

2.—HISTORY AND INCIDENCE OF BANCROFT'S FILARIASIS.

Although discovered in 1493, little effort was made to colonize St. Croix before 1625 and in 1645 there were only 600 inhabitants. In 1650 the island was depopulated by the Spaniards from Puerto Rico. Attempts by the French under the monarchy and subsequently by the Knights of Malta to develop the island failed and it was again deserted in 1720. The Danes obtained possession in 1733 and began importing slaves the following year. Tropical diseases including filariasis could only have become permanent factors in invaliding as late as 1734. The Danes doubtless collected important data during their occupation but these were not available for the present studies. The earliest record we can find of filariasis is in the death returns of the Moravian missionaries* which have been kept since 1844. In that year "rose" (lymphangitis) is

* Information placed at our disposal by the Rev. Dr. JOHN GOERNER, Minister of the Moravian Church in St. Croix.

mentioned and thereafter this condition and elephantiasis appear frequently. NEUMANN (1881) estimated that about 6 per cent. of the population had elephantiasis. Following the acquisition of the Virgin Islands by the United States in 1917, with organization of the Health Department under the U.S. Naval Medical Service, a series of important papers concerning filariasis appeared. BUTLER and HAKANSSON (1917) estimated filarial infection in St. Croix as being 25 per cent. in a population of 14,901. STENHOUSE (1924) found microfilariae in 10 per cent. of bloods examined. HUGHENS (1927) observed microfilariae in 12·5 per cent. of patients in the Christiansted hospital and in 23·8 per cent. of patients in the Fredericksted Hospital. Clinical filariasis as indicated by elephantiasis and hydrocele, common in both towns, was more so in the latter. Under the Civil Administration KNOTT (1936) has made extensive studies on filariasis. In an examination of 2,000 school children, using 1 c.c. of blood in each case, 16 per cent. had microfilariae. At the Old People's Farm, King's Hill, in the centre of the island, using the same quantity of blood from 124 persons he found microfilariae in 46 per cent. At the Civilian Conservation Corps camp, where there were boys from 16 to 25 years of age from all over the island, using 1 c.c. of blood he found microfilariae in 43 per cent. At night, using 20 c.mm. of blood, he found 39 per cent. with microfilariae and studying 10 c.c. of blood he found 53 per cent. with embryos. As to clinical manifestations of filariasis, from personal observation it is clear that all the obstructive and inflammatory phenomena previously seen in Puerto Rico and Antigua are present. There is abundant evidence therefore that filariasis is very prevalent in St. Croix and is probably more so in Fredericksted than in Christiansted. There seems to be a focus of high prevalence at King's Hill (see Table I). There is considerable variation in the incidence of microfilariae noted by different observers and by different methods of study by the same observer (KNOTT). The 10 c.c. studies by KNOTT should, we believe, be considered as the most reliable ; if, therefore, considering his work we roughly estimate the microfilarial incidence of Christiansted as being between 40 and 50 per cent. of the inhabitants, it will be interesting to analyze the degree of infection in mosquito hosts of the same locality.

3.—EXAMINATION OF THE BLOOD OF WILD AND DOMESTIC ANIMALS.

Since filaria larvae found in wild *Culex fatigans* might conceivably come from wild or domestic animals other than man, it was considered advisable to examine the blood of as many, particularly of the former, as possible.

Thus, throughout the studies at various times the blood of large numbers of the following were examined : cats, rats and mice (living trapped specimens), goats, bats (captured alive in their day shelters), fowl, ducks, turkeys and domestic pigeons, the wild mongoose and several species of small lizards. In none of these were microfilariae found.

Christiansted, like all towns in St. Croix, harbours large numbers of mongrel dogs.

An examination of 1 c.c. of blood of thirty-three dogs by Dr. JAMES KNOTT showed that 33 per cent. contained the microfilaria of *Dirofilaria immitis*. It was therefore decided to observe the behaviour of this parasite in two of its reputed insect vectors, namely, *C. fatigans* and *Aëdes aegypti*. That the parasite does not develop readily under experimental conditions in either insect *in St. Croix* was indicated by our efforts to infect them. Using *C. fatigans* fed on a dog with many larvae in the blood, many parasites, all dead, were found next day in old stomach blood, but none had emigrated to the abdomen or thorax. On the other hand amongst the wild *C. fatigans* examined, on two occasions half-grown larvae active and greenish in colour with developed intestinal canal were found near the Malphigian tubes or in the abdomen (Table IV). With experimental *A. aegypti* attempts to get full development failed on many occasions till May, when in two insects fully developed larvae, much shorter than those of *W. bancrofti*, were found in the proboscis on the 15th day. In wild *A. aegypti* young larvae similar to those found in experimental insects were only once found in the abdomen. Considering the large number of dogs in Christiansted and the high percentage of those which have embryos of *Dirofilaria immitis* in the blood, it would appear that although *C. fatigans* and *A. aegypti* may become infected under natural conditions, neither of these insects is the main factor in transmitting the canine infection *in St. Croix*.

There are many horses, mules and asses in St. Croix but most of these are stabled in the country and they were not examined for the embryos of *Setaria equina*.

In wooded and bush country and generally at some distance from human habitations are numerous ground doves, *Columbigallina passerina nigrirostis*. These were found to be heavily infected with a sheathed microfilaria (O'CONNOR, 1936), belonging to a filarial worm, *Vagrifilaria columbigallinae* AUGUSTINE, 1937. A similar microfilaria was rarely found by the writer in the white-headed pigeon, *Columba leucocephala*, the redheaded pigeon, *C. squamosa*, and the mountain dove, *Zenaida zenaida*. Efforts to infect *C. fatigans* with this microfilaria were not successful, and as the four bird hosts mentioned do not nest or roost close to human habitations it is improbable that they would infect domestic mosquitoes within the area of the present studies.

On the whole then, we feel reasonably sure that in our studies with wild mosquitoes we have, with the exceptions already mentioned, been dealing with the microfilaria and larvae of *W. bancrofti*.

4.—DETERMINATION OF THE DURATION OF LARVAL DEVELOPMENT IN *Culex fatigans*.

This was decided by means of 48-hour old imagines fed at night on volunteers in whose blood microfilariae were present. The insects collected next morning after a single blood feed were subsequently kept in mosquito-proofed lantern globes and fed on weak sugar syrup. Each day a few insects were dissected until larvae were found fully developed in the proboscis. The minimum period for this development was found to be : November, 1935, 13 days; December, 1935, to January, 1936, 17 days; and July, 1936, 9 days.

On most occasions with fully developed parasites in proboscis, head, etc., a few parasites which had not completely reached that stage were found in the thorax. The maximum period for larval development was on each occasion 1 to 3 days longer than the minimum figure quoted. The largest number of fully developed larvae found in an apparently healthy insect was thirty-two, distributed as follows : proboscis two, head ten, thorax fourteen and abdomen

six. In addition two parasites, about two-thirds developed, were present in the thorax. These studies made it possible at the different seasons to estimate the phase of parasitic development in wild mosquitoes (see Table I and Fig. 2).

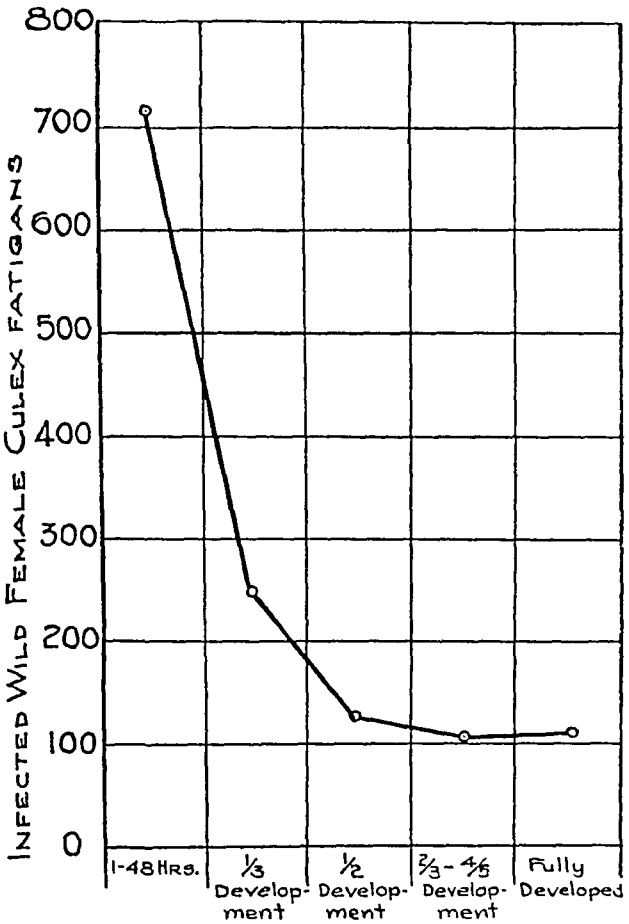


FIG. 2.—Phase of development of *Wuchereria bancrofti* in wild *Culex fatigans*.

5.—Dissection and Examination of 5,000 Wild Female *Culex fatigans*.

Most of the insects were captured in Christiansted.

This city is situated on the north coast at the base of high hills which enclose it on the north-east, east and south, it is thus sheltered from the eastern trade-winds and visitors frequently find it unpleasantly warm ; it is well laid out, most of the thoroughfares running north to south or east to west. The houses are generally well constructed, the better-class ones large and airy, being constructed on the Danish plan, in many of these a high staircase leads to the ground floor which is well above the street level ; even the houses of the poor are generally superior to those of the same classes in such neighbouring islands as Puerto

TABLE I.

RESULTS OF EXAMINATIONS OF FEMALE *Culex fatigans* CAPTURED AT ST. CROIX, VIRGIN ISLANDS.

Number Examined.	With Stomach Blood.	Without Stomach Blood.	Infected.	Per cent. Infected.	Phase of Larval Development.‡					Per cent. Infective.	Insects with two Generations of Parasites.	Insects with Parasites without Stomach Blood.	Insects with Chicken Blood in Stomach.	Chicken Blood in Stomach. Parasites elsewhere.	Location.
					I.	II.	III.	IV.	V.						
929	535	394	430	46·2	316	69	28	12	28	3	22*	90	81	8	Inside H. P.'s house.†
252	170	82	49	19·4	17	13	13	6	6	2·3	5*	15	26	2	Outhouses, rain barrels of H. P.'s house.†
336	291	45	119	35·4	84	25	7	5	5	1·4	7	10	27	0	Inside V. H.'s house.†
362	234	128	46	12·7	20	7	10	6	5	1·3	2	8	72	0	Outhouses, rain barrels of V. H.'s house.†
669	464	205	103	15·3	44	32	14	13	6	0·9	6	25	14	0	Municipal Hospital, Christiansted.
2,339	1,453	886	462	19·7	226	107	65	53	48	2·05	36*	126	203	10	Town of Christiansted, houses, outhouses, rain barrels.
107	80	27	42	39·2	14	6	1	8	17	15·8	4	5	2	0	Old Peoples' Farm, Kings Hill.
6	6	0	3	50	2	0	0	1	0	0	0	0	0	0	Town of Castle Coakley.
5,000	3,233	1,767	1,254	25·08	723	259	138	104	115	2·3	82	279	427	20	Totals.

* One mosquito with three generations of *W. bancrofti* larvae.

† One occupant of each house was known to have microfilariae in the blood.

‡ As the length of each phase varies according to atmospheric conditions at different seasons they are here classed as follows :—

Phase I. From sheathed microfilaria till parasites begin to shorten in thorax.
Phase II. Larvae about one-third developed.
Phase III. Larvae about half grown.
Phase IV. Larvae about two-thirds to four-fifths grown.
Phase V. Fully developed larvae.

Rico and Antigua ; most have a yard of varying dimensions with one or more outhouses for fowls, toilet and working tools, etc. The natives are almost entirely dependent on rain for drinking water which is collected in unguarded, wooden or metal barrels in the vicinity of each house ; in these receivers most of the *C. fatigans* and *A. aegypti* breed. Other sources for the breeding of culex are surface wells with brackish water used for washing and cleaning, pools near the coast with brackish water when after rains the diluted water becomes suitable for breeding, cisterns, tanks, unused sugar kettles, gasoline and other tins, broken bottles and cans in rubbish heaps. Breeding also occurs in damaged or obstructed gutters and water spouts of the roofs, and inside houses in disused tubs, pails and vases (HAYES, 1930).

SOURCE OF THE INSECTS.—*Culex fatigans* was collected from the following places :—

A.—In Christiansted.

1. Inside the house of H. P. (occupant known to have microfilariae).
2. Outhouses in yard of same.
3. Inside house of V. P. (occupant known to have microfilariae).
4. Outhouses in yard of same.
5. Inside Municipal Hospital.
6. A miscellaneous group taken from houses and outhouses in every part of the city regardless of information as to parasitization of the human population.

B.—Outside Christiansted.

7. Old People's Farm at King's Hill (centre of the island).
8. Castle Coakley.

Although many mosquitoes were taken at 1, 2, 3, 4 and 7 where one or more persons were known to have microfilariae in the blood, yet in the hospital group with a constantly moving population parasites were frequently not found for long periods in any of the insects. In the miscellaneous town group large numbers of insects were collected from houses where probably, and in some instances (from observation) certainly, no persons had microfilariae. It is felt therefore that the study represents a fairly practical cross section of the mosquito population of the city.

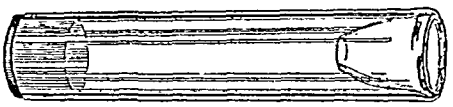


FIG. 3.—Pyrex glass mosquito catcher.

The insects were captured in ordinary pyrex glass mosquito catchers (Fig. 3) between 7.30 and 8.30 a.m. daily. After labelling the dishes with the place of capture they were brought to the laboratory and the mosquitoes were at once partially stunned with tobacco smoke. The wings were removed and they were put aside in dry Petri dishes. Working between 9 a.m. and 5 p.m.

it was found possible, one person dissecting and one making the microscopic examinations and recording results, to study between seventy and seventy-five daily. The head and proboscis, the thorax, and the abdomen respectively were examined on the same microscopic slide in three separate drops of sterile saline. Only insects with blood in the stomach or with mature eggs, as evidence of a previous meal, were studied. The results of the studies are shown in Tables I and II.

TABLE II.

INFECTION OF WILD *Culex fatigans* WITH THE LARVAE OF *W. bancrofti*.
SEASONAL INCIDENCE.

Month.	Number. Examined.	Number Parasitized.	Percentage Parasitized.	Number Infective.	Percentage Infective.	Percentage of Parasitized which are Infective.
1935.						
October	315	76	24·1	5	1·5	6·5
November*	1,367	317	23·1	42	3·07*	13·2
December*	517	160	30·9	10	1·9	6·3
1936.						
January	484	139	28·7	5	1·03	3·59
February	157	53	33·7	3	1·91	5·66
March	38	4	10·5	0	0	0
April	16	1	6·25	0	0	0
May	131	24	18·3	0	0	0
June	1,005	293	29·15	25	2·48	8·5
July	635	95	14·9	16	2·51	16·8
October	23	4	17·3	0	0	0
November	88	31	35·2	1	1·13	3·19
December	224	57	25·4	8	3·6	14
Totals	5,000	1,254	25·08	115	2·3	9·1

* In considering the high percentage of parasitized insects it should be noted (see Table I) that all the King's Hill dissections were made during these two months.

6.—EXAMINATION OF OTHER WILD MOSQUITOES.

Aëdes aegypti.—In this series 386 female insects were studied between 5th October, 1935, and 15th January, 1936. One hundred and ninety, or 49 per cent., were found to be infected with the microfilariae or young larvae of *W. bancrofti.* Small infections predominated and only in ten individuals were more than twenty parasites found. The largest number recorded were fifty-four in the thorax of one and sixty-six in the thorax of another taken in the house of H. P.

Under natural conditions microfilariae exsheath rapidly in *Aëdes* and quickly reach the thorax, for their greatest number were generally found there, a conclusion confirmed when insects were fed on volunteers with microfilariae in the blood. In such insects most parasites had left the stomach within a few hours, and those left in it dead or dying were very few compared with the numbers usually observed in *C. fatigans*. After leaving the stomach of *Aëdes* the parasites are found in the thorax, and were not observed developing in or near the Malphigian tubes or elsewhere in the abdomen. On arrival in the thorax many parasites die even before shortening; survivors may remain active and unshortened for several days; on the other hand shortening may take place early. In experimentally fed insects, parasites, shortened and sluggish or active and unshortened, have been found alive in the thorax as late as nine days after infection, but in no case did development of the intestinal canal take place. After this time only dead parasites were found in the thorax up to the 13th day. Dead and degenerating parasites were found in the thorax of sixty-eight of the wild *A. aegypti*. In degenerating parasites granulation occurs and is followed by fragmentation. Whether living or dead the parasites did not apparently affect the mosquito host and amongst the experimentally fed insects the mortality was much lower than was the case when using *C. fatigans*. Parasites were found in large, medium and small varieties of *A. aegypti*. In no instance was chicken blood found in the stomach of these, and efforts to make these imagines feed on blindfolded chickens in cages were invariably unsuccessful in St. Croix.

The high incidence of infection of wild *A. aegypti* seems related to the biting habits of the insect which, being shy and easily disturbed, has been observed to bite several or more individuals in the same room within a few minutes. It is thus more liable to acquire parasites than *C. fatigans* which more commonly feeds to repletion on one person. The small infections usually found in *A. aegypti* are due in part to the fact of the smallness of the interrupted feeds, but even more so to its feeding more commonly during those hours of the day when fewer parasites are present in the peripheral blood of the human host. As noted, the larvae never reached the infective stage.

Anopheles albimanus.—KNOTT and BEATTY in 1935 found that in St. Croix *A. albimanus* was, under experimental conditions, a suitable host for the larval phase of *W. bancrofti*; during the present studies this observation was confirmed. In *A. albimanus* imagines fed on 9th October, 1935, fully developed larvae were found in the proboscis on the 27th day of the same month, development being apparently a little slower than in *C. fatigans*; in one out of ten wild *A. albimanus* studied, young very active *W. bancrofti* larvae were found. In St. Croix *A. albimanus* has only been found in limited areas; it has sometimes become so prevalent as to cause severe outbreaks of malaria, but with the effective methods of the Public Health Service under Dr. KNOTT's administration it has been easily controlled, so that it was difficult to procure even a few specimens during these studies. For this reason in St. Croix it cannot under ordinary conditions be considered of major practical importance as a vector of filariasis, although because of malaria vigilance is always necessary.

Culex habilitator was likewise shown by KNOTT and BEATTY in 1935 to be a good laboratory vector for the larvae of *W. bancrofti*. In the present studies development of the parasites in this insect was of the same duration as in *C. fatigans*. Throughout the year, however, the insect was never found in very

great numbers and so, though contributory, is not considered very serious as a transmitting agent of filariasis. Parasites varying in development between one and seven days were found in four out of five wild *C. habilitator* which were captured.

Aëdes taeniorhynchus.—This insect was collected four times in houses near the seashore at Christiansted. Young parasites, dead and degenerated, were found in two of the insects.

7.—The Fate of Microfilariae and Larvae in and after leaving *Culex fatigans.*

During, and soon after feeding, sheathed microfilariae are passed by *C. fatigans* per rectum, and sheaths and subsequently exsheathed larvae are found in the droppings until 24 hours later. This was demonstrated by placing newly fed mosquitoes in containers made by apposing microscope slides held together by adhesive tape (Fig. 4).* The slides were subsequently separated,

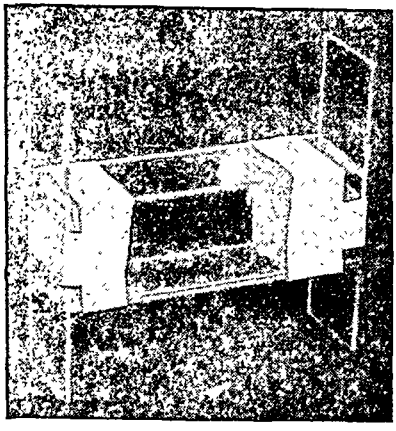


Fig. 4.—Container made from microscope slide for collection and examination of fly droppings.

fixed and stained with haematoxylin when the parasites were demonstrated in the droppings. Many droppings were washed off in efforts to fix them, so it was not estimated how many parasites were lost, but from the numbers found it is clear that many succumb in this way.

* Photographs of such boxes and demonstration of the slides which made them up were first shown by me for Professor O'Connor at this Society's Laboratory Meeting in March, 1936.—Clayton Lane.

In fifty-five instances only dead parasites were found in the stomach blood, none having reached the thorax. These were generally small infections but ten or more such parasites were found in seven insects, the highest recorded being twenty-six on two occasions.

TABLE III.

FATE OF LARVAE OF *Wuchereria bancrofti* IN WILD *Culex fatigans* DURING THE FIRST 20 HOURS AFTER AN INFECTIVE FEED.

	50 Mosquitoes dissected from 4 to 16 hours after an Infective Feed.						50 Mosquitoes dissected from 16 to 20 hours after an Infective Feed.					
Blood ...	Red : little clotting.						Dark : much clotting.					
	Number of Larvae.						Number of Larvae.					
	Total.	Some variations.					Total.	Some variations.				
In stomach												
Dead	289	0	1	8	123	12	591	55	51	67	18	18
Feeble	71	0	0	0	0	12	92	5	2	1	0	17
Active	590	50	25	25	17	0	8	0	0	0	0	1
In thorax	596	56	72	31	13	1	643	25	63	1	58	44
Grand total	1546						1,334					

Table III shows that of 2,880 parasites found in 100 wild culex (others had doubtless been passed previously in droppings) 880 or 30·5 per cent. died in the stomach blood ; 163 more parasites described as " feeble " should be added to this loss since they could not possibly in this condition extricate themselves from the clots which become larger and more numerous from 12 hours after the insect feeds. That such feeble parasites do not reach the thorax will be appreciated by comparing Parts I and II of the Table. Thus, if these parasites are added to those already dead, then of 2,880 parasites found 1,043 or 36·2 per cent. would eventually have succumbed without further development.

Having " exsheathed," a comparatively small number of larvae pierce the stomach wall and travel through the abdomen to the thorax which they may reach as early as two hours after the insects feed. The majority, however, as shown by IYENGAR (1936) and O'CONNOR and BEATTY (1936) reach the thorax later by way of the anterior midgut which they penetrate. Since parasites reach the thorax from 2 to 24 hours after the infective feed, the great variation in size, shape and motility at the latter time is explained, for some will have already

become shortened, broad and sluggish while others of the same generation will be long, narrow and very active. For the first few days after feeding there was sometimes a heavy mortality of insect hosts while the survivors showed a fair number of parasites, all dead, in the thorax; on other occasions the insects themselves were apparently healthy when killed 24 hours after the feed but all the parasites in the thorax were dead both in heavy and light infections. This shows that, even in a known suitable insect vector, parasites which have reached the thorax do not invariably survive. On two occasions exsheathed larvae were found in the proboscis of the mosquito 24 hours after ingestion; these apparently had wandered out of the thorax while seeking a suitable location for development. After the first two days few parasites seem to die in the mosquito. No parasites were found developing except in the thorax until about 2 days before full development was reached; then, however, even before the tail papillae were recognizable a few parasites were found in the abdomen, while others from the thorax exhibited definitely progressive movements.

Although 25·08 per cent. of *C. fatigans* had become " infected " only 9·1 per cent. of these contained fully developed or " infective " parasites; while of 5,000 insects studied only 2·3 per cent. contained fully developed, infective larvae (Table II).

Probably, however, the percentage of *C. fatigans* which actually infect man in Christiansted is much smaller, as the following considerations suggest.

I.—A number of insects are killed by strong wind, sudden torrential rain, spiders, bats and small lizards. Living, dying and dead larvae may be found in drowned insects. Chickens probably destroy many. In a first experiment to see if *C. fatigans* would feed on chickens, the mosquito-proofed cage used was so small that the mosquitoes could not get out of reach of the bird, and next morning every one of several dozen had disappeared; the next evening the chicken placed in the same cage was blindfolded with an adhesive tape bandage and the following morning all the insects had not only survived but their stomachs contained chicken blood. Furthermore, chickens wandering into the laboratory were observed devouring culex and aëdes resting in the dark corners of the room.

II.—Both mosquito and parasites may be killed by mutilation while biting. Experimentally, when insects with mature larvae were allowed to bite man and crushed by a blow as soon as the sting was felt, it was found that while some parasites survived others were either killed outright or so damaged as to prevent their further activity. It is possible that when so crushed some parasites, surviving, might make their way from thorax or abdomen to the human skin, yet probably more succumb than survive by such treatment.

III.—The bite of *C. fatigans* is immediately irritating to a person who is awake, and scratching by the victim results, so that some parasites having reached the skin are damaged or killed while wandering on or entering it.

IV.—Parasites may be deposited on an unsuitable host. It has been shown that culex will readily bite domestic fowl. Moreover, from the frequency with which developing *W. bancrofti* larvae were found in the thorax of insects with chicken blood in the stomach it appears that *C. fatigans* is almost as partial to this kind of avian blood as it is to human blood. If *W. bancrofti* larvae are fully developed in a mosquito biting a fowl it is probable that the parasites get on to the skin or get lost among the feathers of the bird.

V.—Parasites may leave the proboscis as the result of stimuli other than that of contact between mosquito and the skin of any animal. While separating the head of *C. fatigans* from the thorax *without touching the proboscis*, larvae were observed fairly frequently to leave the tip of the latter and enter the drop of saline in which the parts were lying. The method

used for making permanent preparations of " infective " mosquitoes was as follows. The insects were partially stunned by tobacco smoke and allowed to drop into hot Schaudinn fixing fluid. The wings were only removed after fixation and prior to mounting. Nevertheless, on several occasions larvae were found fixed in the act of leaving the proboscis (Fig. 5). In many of the small houses of the poor people the atmosphere is thick with



FIG. 5.—Fully developed larva of *W. bancrofti* leaving proboscis of *C. fatigans*. × 100.

smoke from tobacco or stoves and it is probably that larvae leave the proboscis of resting mosquitoes under such conditions. Whether mature larvae require any stimulus whatever to entice them from the proboscis would be well worth investigating. Once arrived there and with an available exit before them there seems to be no reason why they should not, by their wriggling and progressive movements, work their way to the exterior.

Apart, however, from this last mere speculation there is abundant reason for believing that less than 1 per cent. of *Culex fatigans* in Christiansted succeed in so depositing their larvae as to insure the latter entering their definitive host, man.

8.—Distribution of Fully Developed Larvae in *C. fatigans*.

During previous studies in different countries and in experimentally fed insects it was frequently observed that fully developed larvae may be found in different parts of the body of the mosquito. In the present instances this was closely studied in ninety-two of the infective *Culex* considered in Table IV. It is probable that such parasites in the thorax are " waiting their turn " for entry to the already occupied head and proboscis, but why 57, or 62 per cent., of the parasites should wander to the abdomen seems worth inquiry. A few parasites might aimlessly find their way to this locality but that so many do so suggests that this phenomena is not accidental. It is possible that for some reason the insects leave the thorax just before or on becoming fully developed

and go to the abdomen, until after going back to the thorax they can finally reach the head. Such parasites in the abdomen were carefully observed to see if they ever tried to pierce the tissue at the posterior end of the insect but no suggestion of an effort to do so was observed. It does seem possible that some larvae in

TABLE IV.

DISTRIBUTION OF LARVAE OF *Wuchereria bancrofti* WITHIN 92 *Culex fatigans* FOUND ON CAPTURE TO BE INFECTED.

Site of Infection.	Number of Mosquitoes Infected.	Number of Larvae in the Developmental Phases.*					Greatest Number of Larvae in any one Mosquito.
		I.	II.	III.	IV.	V.	
Proboscis	37					50	2
Head	27					39	3
Thorax	7	33					12
	10		36				12
	6			32			16
	25				80		21
	47					95	11
Abdomen	36					57	4

* The larvae in 70 of these mosquitoes were assigned to one infective feed; in 19 to two, and in 3 to three infective feeds. In two mosquitoes certain larvae appeared to be those of *Dirofilaria immitis*; in three the stomach held bird's, probably chicken's, blood; in four, all previously infected, the stomach held fresh blood with microfilariae still in it.

the abdomen of the mosquito might, if the insects themselves were crushed during biting, make their way through ruptures of the insects to the human skin. While five parasites almost fully developed were found in the thorax of the same insect, the largest number of fully developed larvae found in a wild culex was 14, distributed thus—proboscis 2, head 5, thorax 6, abdomen 1.

9.—VARIATIONS IN THE INCIDENCE OF INFECTION IN *Culex fatigans*.

It has been demonstrated that in and about certain habitations, where one or more persons have fairly high numbers of microfilariae in the blood at night (H. P., V. H. and Old People's Home, Tables I and II), the insects are frequently heavily infected. It remains to consider the degree of insect infection at the

Municipal Hospital with its constantly changing population and also the miscellaneous group from Christiansted. The microfilarial incidence of the general population had not been estimated at the time of these studies but much was learned from the study of the mosquitoes. Not only were insects examined from many parts of every street in the city but the collections were made again and again from the same places. Considering fifty parasites as heavy infections in the whole series, the distribution of such will be easily appreciated by examining Table V.

TABLE V.

LOCALITIES IN WHICH THERE WERE FOUND MORE THAN 50 LARVAL *Wuchereria bancrofti* IN A *Culex fatigans*.

Place.	Number Examined.	With 50 or more Parasites. Number.	Per cent.	Number of Parasites in each Mosquito.
Inside house H. P.	929	17	1·8	141, 69, 67, 55, 153, 60, 76, 63, 106, 85, 98, 63, 54, 116, 121, 119, 80.
Outhouses, rain barrels H. P.	252	4	1·6	111, 54, 52, 144.
Inside house V. H.	336	6*	1·8	80, 61, 67, 62, 72, 72.
Outhouses, rain barrels V. H.	362	0*	0	
Municipal Hospital	464	2	0·43	67, 90.
Christiansted	2,339	16	0·69	88, 52, 28, 76, 64, 318, 82, 63, 53, 111, 119, 60, 50.
Old People's Farm	107	1	0·93	74.

*Residence and outhouses fairly well separated on top of hill in open clearing.

With regard to the town of Christiansted, subsequent analysis of this group showed that all the high counts except three came from one house in which the occupant, A., was later found to have a high microfilarial count. Thus, except for this house only three large counts were found in insects from the rest of the city. Similarly it was found throughout the studies that the highest incidence

of infected and infective mosquitoes were found in the same locations (except the Old People's Farm) in which counts ranging from fifty to about 150 parasites were found most commonly in wild *Culex*. These locations are therefore of major importance, both as disseminating foci of *Wuchereria bancrofti* infection and from the point of view of reinfection of the patient and his living companions. If the locations where there is evidence of heavy infection of mosquitoes are investigated it will be found that other factors have to be considered in determining the danger of the spread of filariasis.

The highest incidence of infection of *C. fatigans* was found at the Old People's Farm where Dr. KNOTT found microfilariae in 46 per cent. of 124 people examined. Here obviously infection is spreading amongst the small group of occupants but, as the farm is situated on a hill in the middle of the island (Fig. 1) and far from the populated sections, there is little likelihood of filariasis spreading from this to other neighbourhoods. At the Municipal Hospital on a high hill behind Christiansted, with a constantly changing population it is probably not disseminated to any great extent especially since few of the patients remain long enough to acquire heavy infections. In the town generally, while small infections are disseminated everywhere, mosquito studies indicate that the more serious menace is from certain houses like those of H. P., V. H. and A. With this information certain facts may be considered with a view to preventing or at least reducing the prevalence of filariasis.

1. It would be impracticable to kill all or appreciably reduce the number of transmitting mosquitoes. Most of the *C. fatigans* breed in rain barrels on which the population mainly depends for its drinking water since there are neither rivers, ponds nor suitable wells with potable water. Larvicidal fish have been distributed freely for use in such barrels and are undoubtedly useful, but with limitations. When the larvae are killed, the stronger may devour the weaker fish and the supply dwindles. Moreover, the population are careless in maintaining these or requisitioning further supplies when necessary. The treatment of water in rain barrels and other receptacles with oil or Paris green is obviously unsuitable. The screening of all rain barrels and other receptacles has been considered but the enormous amount of material that would be required, the frequent repairs necessary owing to wear and careless handling by the people, and the fact that many receptacles could not be properly screened indicate that this measure would not prove successful. Daily destruction of mosquitoes in houses and outhouses might be tried but this measure, requiring considerable supervision of personnel, has not been found applicable in large towns.

Adoption of any of the foregoing measures would entail a large staff of inspectors or supervisors on constant service for indefinite periods at a cost beyond the power of government or municipality to maintain.

It seems, therefore, that with our existent lack of knowledge we have no method for application which would insure the destruction of all insect carriers

and therefore the complete prevention of filariasis. It remains to be seen whether short of such an ambitious project something might not be done to limit appreciably the prevalence of filariasis and so (believing that much reinfection is necessary to produce symptoms), to reduce the degree of such reinfection and therefore the severity of the manifestations.

The present work shows that, while some degree of infectivity of wild *Culex fatigans* is found almost everywhere in Christiansted, foci of high *infectivity* of the insect are not many. From family incidence studies reported in other contributions as well as from the present work, it is clear that the greatest number of cases and the most severe ones occur in single houses or groups of houses. Such foci are easily detected by studying the microfilarial incidence in man in all houses in conjunction with the infective mosquito incidence. It would seem that just as in dealing with hookworm and ascaris, methods of prevention are concentrated especially on families with heavy infections, so preventive measures on filariasis should be especially directed against houses with high microfilariae numbers and high insect *infectivity* incidence. In Christiansted up to the present, high insect infectivity was only observed in three houses. While a few more may be subsequently discovered the indications are that less than a dozen houses would require preventive measures of an intensive nature.

10.—Studies and Methods Advocated with a View to Reducing Bancroft's Filariasis in a Town such as Christiansted, St. Croix.

A. The general measures adopted should be as follows :—

I.—The incidence of persons with microfilariae should be determined at the same time for the whole population.

II.—The percentage of *infective* mosquitoes should be determined in the same houses and outhouses, etc. The mosquito " infective " incidence may be more valuable than the microfilarial incidence, partly because while some natives do not readily submit to having blood taken from them, yet when the reasons are explained to them they rarely object to their mosquitoes being collected. Furthermore, a person with microfilariae having been infected in another locality may be in a place where there are few or no mosquitoes and so will not be a serious menace. On the other hand, the repeated finding of infected mosquitoes is proof positive that one or more persons with microfilariae is near by.

III.—These studies might well be repeated about every three years.

B. In houses of high human and mosquito infectivity incidence the following local measures should be carried out :—

I.—The nature of filariasis, its transmission and prevention should be completely and simply explained to the occupants of the house where control measures are instituted.

II.—Suitable containers for potable and other water supplies should be adequately screened with wire netting. Where containers are not suitable they should be replaced.

III.—The use of the mosquito net should be demonstrated. (If the occupants cannot afford them these should be provided from public funds.)

IV.—The proper maintenance and use of all screening should be supervised at intervals by the existing sanitary officers.

V.—When possible occupants should be encouraged to keep fowls in their yards *near* the house.

VI.—The number of mosquitoes in the houses and the percentage of these which are infective should be recorded from time to time in order to evaluate the results of preventive measures.

VII.—Efforts to have adult mosquitoes killed daily by the inhabitants while highly desirable will usually be found impracticable. This measure would be too expensive for government maintenance, but where full co-operation is assured should be adopted to supplement the foregoing.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

MECHANISM OF OEDEMA IN HELMINTHIC ANAEMIAS.

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The occurrence of oedema in helminthic anaemia is not uncommon. The fact that the association is variable suggests that more than one factor plays a role in its production. In order to determine the causative factors, twenty-two cases of helminthic anaemia with oedema have been subjected to special clinical and biochemical investigations and the effect of various diets and therapeutic measures on the conditions were observed.

The following observations and findings may throw light on the mechanism and significance of this associated oedema.

1. Although all cases showing oedema are markedly anaemic (haemoglobin below 50 per cent.) yet there is no constant relation between the occurrence and degree of the oedema and the severity of the anaemia, e.g., many cases having haemoglobin values of 10 to 20 per cent. showed no evidence of oedema. This suggests the presence of other factors beside the anaemia in its production.

2. Oedema most commonly occurs in anaemia due to ancylostomiasis and intestinal bilharziasis. In the latter it is more common in the dysenteric cases. These two infections are the ones which give rise to marked disturbance of the gastro-intestinal tract and to marked anaemia.

3. The oedema is usually soft, pale and following gravity in distribution; mostly limited to the lower half of the body, it may occasionally acquire a generalised character (four out of twenty-two cases).

Ascites did not occur as a part of the general oedema except in three cases in which a moderate degree of cirrhosis was present. These cases do not include those associated with portal obstruction or polyserositis.

4. Clinical examination as well as blood pressure and pulse estimations have excluded a purely cardiac origin of this oedema. There was a tendency to slightly low figures of both systolic and diastolic pressures in most cases; in some cases there was dilatation of the heart (general, not limited to the right heart; without evidence of venous engorgement, thus excluding beriberi). These findings suggest a certain degree of circulatory weakness but no actual failure.

In three cases the oedema disappeared, within 3 or 4 days after admission, on a protein-free diet, suggesting that rest was the main cause of its disappearance and that circulatory fatigue had been an important additional factor in its production.

5. With the exception of diminished deep reflexes in some cases, there was no evidence of peripheral neuritis suggesting avitaminosis B_1 in any case.

6. Albuminuria is absent in these cases, with the exception of the slight albuminuria produced by bilharziasis. Blood urea estimations and urea concentration tests were within normal limits in all cases. These findings together with the low blood pressure and low blood cholesterol are definitely against any nephritic or nephrotic factor in the causation of this oedema. These observations do not agree with those of DE LANGEN and EERKENS (1933) who found a combination of nephrosis and ancylostomiasis in seventeen out of twenty cases in Java. They concluded accordingly that there is an aetiological relationship between the two diseases. Such a conclusion cannot be accepted for the following reasons :—

(*a*) No mention has been made of the degree of infection in these cases. From the haemoglobin values it can be seen that the anaemia was mild (above 60 per cent. in sixteen cases) and, therefore, not sufficient to produce an intoxication severe enough to result in nephrosis.

(*b*) The frequency of *Ancylostoma* infection in an endemic area results in its association with many diseases. An aetiological relationship based only on such an association especially in an endemic area could not be safely accepted without further proof.

(*c*) We met with a case (outside this series) whieh showed the typical picture of nephrosis (albumin, fatty, hyaline and epithelial casts without nitrogenous retention, or circulatory disturbances and high blood cholesterol, 785 mg. per cent.).

When this case was followed, signs of a nephritic process were detected 6 months later (nitrogen retention, rise of blood pressure to 170/120, appearance of retinal changes of albuminuric nature) thus the case proved to have been in the nephrotic stage of nephritis when first seen.

This case shows the importance of following up cases showing the nephrotic syndrome over long periods in order to detect signs of renal insufficiency of inflammatory origin. The absence of these signs is to be accepted as the final proof of their purely degenerative nature.

BIOCHEMICAL INVESTIGATIONS.

Investigations on the blood chemistry of these cases have been carried out in fifteen cases ; the following findings were obtained and will be discussed :—

1. The role of *serum calcium* in diminishing capillary permeability has led us to study its concentration in such cases. It was found to be slightly diminished in seven out of seventeen cases. It is to be noted that these cases with hypocalcaemia showed hypoproteinaemia as well.

The cause of this hypocalcaemia cannot be attributed to either deficient supply, as the food of the hospital class of people is rich in calcium (milk, cheese); or to deficient absorption, as vitamin D is also available in milk while the exposure to sun helps the ultra-violet rays to synthesise still some more. Moderate hypocalcaemia is observed in patients with chronic malnutrition especially if advanced to the stage where oedema develops (JANSEN, 1919).

Moreover, it appears to be established that the presence of an adequate amount of protein in the serum is one of the conditions essential for the maintenance of the normal calcium concentration. The fact that the seven cases with hypocalcaemia in our series were associated with hypoproteinaemia supports our conception that the hypocalcaemia here is secondary to the hypoproteinaemia and not a primary nutritional deficiency.

That this hypocalcaemia may have something to do with the occurrence of oedema in these cases was shown by the fact that injections of calcium (in addition to the high protein diet) did hasten the disappearance of the oedema in three cases.

2. The determination of the *plasma proteins* showed low values in 12 out of the 15 cases analysed.

STARLING (1896) showed that the exchange of water between the blood and tissues depends on a balance between the colloidial osmotic pressure of the plasma proteins and the hydrostatic pressure within the capillaries. The significance of hypoproteinaemia in the production of nephrotic oedema has been suggested by EPSTEIN (1917) and confirmed by other observations. There is no reason why a similar mechanism may not be responsible for the production of various oedemas of obscure origin. This induced us to investigate the total plasma proteins as well as the albumin-globulin ratio in these cases.

Although the total proteins were not greatly below normal limits, the albumin fraction was unusually low. In anaemia without oedema hypoproteinaemia of a lesser degree has occasionally been found (three cases). Such cases, if left for some time, will certainly pass into the oedematous group.

TABLE.
SERUM PROTEINS.

Hospital Number.	Total Protein.	Albumin.	Globulin.	Albumin/Globulin Ratio.	Calcium.
4562	5·44	3·63	1·81	2	9
4681	6·31	2·56	3·75	0·78	7·5
4911	6·12	2·56	3·56	0·71	9·7
5726	6·03	3·2	2·83	1·13	10·3
4420	6·15	3·73	2·42	1·54	8·38
4792	5·02	1·89	3·13	0·7	7·7
3971	6·73	3·58	3·15	1·13	8·25
3948	5·59	2·15	3·44	0·77	8·41
5121	6·72	2·28	4·44	0·54	9·1
3061	6·32	3·76	2·56	1·46	8·7
5619	5·95	3·07	2·88	1·41	7·97
5815	5·62	2·82	2·8	1	9·9

Results are expressed in grammes of proteins per 100 c.c. serum. The average serum proteins in normal healthy Egyptians, free from parasitic infections, were found to be : Total proteins, 7·13 ; Albumin, 4·49 ; Globulin, 2·64 ; Albumin/Globulin ratio, 1·7.

Twelve cases showing hypoproteinaemia with albumin reduction, disturbed albumin/globulin ratio and hypocalcaemia.

6. Albuminuria is absent in these cases, with the exception of the slight albuminuria produced by bilharziasis. Blood urea estimations and urea concentration tests were within normal limits in all cases. These findings together with the low blood pressure and low blood cholesterol are definitely against any nephritic or nephrotic factor in the causation of this oedema. These observations do not agree with those of DE LANGEN and EERKENS (1933) who found a combination of nephrosis and ancylostomiasis in seventeen out of twenty cases in Java. They concluded accordingly that there is an aetiological relationship between the two diseases. Such a conclusion cannot be accepted for the following reasons :—

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as well as loss of blood in the stools (ankylostomiasis). Similar observations have been reported in gastro-enteritis in children with subsequent oedema by ASHBY and MOSCHCOWITZ, and in repeated small bleedings into the intestine by MOSCHCOWITZ (1933).

(*d*) There is little in the literature to indicate whether the anaemic state itself is capable of producing hypoproteinaemia. Hypoproteinaemia has been demonstrated in Addisonian anaemia by KAHN and BARSKY (1919) in three cases and by MEULENGRACHT (1927) in twelve cases. MEULENGRACHT maintains its possible significance in the production of oedema in this disease. Here the hypoproteinaemia has been ascribed either to deficient blood formation or to excessive blood destruction; but we cannot exclude the possibility of deficient absorption of proteins (constant achylia, diarrhoea, etc.) in this disease as well, as there is no evidence in the literature of any investigations carried out on these lines. PETERS, EISENMAN and BULGER (1925) found low proteins in three out of seven cases of profound anaemia; in all moderate oedema was present, but no mention of the mechanism of the hypoproteinaemia in these cases was made.

(*e*) Occurrence of *ascites in bilharzial cirrhotic cases* tends to aggravate the degree of hypoproteinaemia as some of the serum proteins will be lost in the ascitic fluid.

(3) *Chlorides of the whole blood* estimations did not show any alteration suggestive of their implication in the causation of the oedema in these cases. The increase of the whole blood chlorides found in this series is attributed to preponderance of plasma chlorides over cell chlorides and not to actual increase in the former.

4. *Cholesterol* content of the blood was diminished in all the cases examined, the lowest figure being 54 mg. and the highest 133 mg. Seven controls without anaemia from the same class of patients showed figures of 150 to 180 mg. per cent., suggesting that this reduction is related to the anaemia present, and not to any extrinsic dietetic factor.

5. There remains a group of three cases where in the blood chemistry no changes were met with that could be incriminated in the causation of the oedema; and in which the oedema did not disappear until the anaemic condition improved under anti-anaemic therapy. This group demonstrates that the *anaemic state* may be the most important factor in the causation of oedema.

THERAPEUTIC INVESTIGATIONS.

1. In three cases the oedema disappeared within 2 to 4 days after admission on protein-free diet, suggesting that rest was the main factor in its cure and that circulatory fatigue is an important factor in its production.

2. In sixteen cases the oedema disappeared on protein diet within 10 to 15 days after admission before anything had been done to combat either the anaemia or the parasitic infections.

The final confirmation that hypoproteinaemia was the main factor in the production of oedema in these twelve cases was given by the fact that their oedema disappeared on full hospital diet with additional proteins within 10 to 15 days after admission before any therapeutic measures to combat the anaemia or the parasitic infection were taken. Nevertheless, the anaemic state seems to be important as a factor participating in the causation of the hypoproteinaemia as well as predisposing to an easier exchange of water possibly by increasing the permeability of the capillary walls through malnutrition or by other alterations in the physico-chemical character of the blood (viscosity, osmotic pressure, etc.).

The slight degree of reduction of the plasma proteins would by itself be unable to produce oedema in the absence of anaemia. The possible factors responsible for the production of hypoproteinaemia in these cases are discussed in the following :—

(*a*) *Insufficient intake of proteins.*—Hypoproteinaemia was demonstrated in war oedema by SCHITTENHELM and SCHLECHT (1918), JANSEN (1919), NIXON (1920) but they did not ascribe it to the hypoproteinaemia. Later experimental work has shown the relation between protein-poor diet and oedema. DENTON and KOHMAN (1918) found that rats fed on a diet of carrots develop oedema, but if the diet contained an adequate supply of proteins no oedema occurred; SHELBURNE and EGLOFF (1931), caused hypoproteinaemia in a dog by reducing the protein intake, oedema only appearing when the plasma proteins reached 3·2 grammes per cent. on the 83rd day. MAVER (1920) reported oedema in oxen and horses fed exclusively on distiller's wash, the total amount of protein in this foodstuff being 0 to 5 per cent.

That the diet of our hospital class of patients contains a small amount of proteins is manifested by the fact that some of them reported having had their last meat meal 2 years ago while scarcely any eggs are consumed. To consider deficient protein intake as a contributing cause of the hypoproteinaemia and oedema in this class of patients is justified, i.e., this oedema is in part if not entirely nutritional.

(*b*) *Insufficient absorption of protein.*—A certain degree of enteritis is a common sequel of intestinal parasitism (bilharziasis and ancylostomiasis) and may interfere with the absorption of food proteins while the concomitant diarrhoea may hurry the passage of the intestinal contents before absorption is complete. Evidence of the occurrence of impaired absorption is found (*a*) in the presence of undigested muscle fibres in the stools in the diarrhoeic cases ; (*b*) in the glucose absorption curve as compared with intravenous glucose. In fourteen of these cases there was evidence of delayed absorption.

Achlorhydria or hypochlorhydria may also be responsible for the incomplete digestion of proteins.

(*c*) *Excessive loss of proteins* can occur in the diarrhoeic cases especially if dysenteric in character (intestinal bilharziasis) through loss of serum proteins

as well as loss of blood in the stools (ankylostomiasis). Similar observations have been reported in gastro-enteritis in children with subsequent oedema by ASHBY and MOSCHCOWITZ, and in repeated small bleedings into the intestine by MOSCHCOWITZ (1933).

(*d*) There is little in the literature to indicate whether the anaemic state itself is capable of producing hypoproteinaemia. Hypoproteinaemia has been demonstrated in Addisonian anaemia by KAHN and BARSKY (1919) in three cases and by MEULENGRACHT (1927) in twelve cases. MEULENGRACHT maintains its possible significance in the production of oedema in this disease. Here the hypoproteinaemia has been ascribed either to deficient blood formation or to excessive blood destruction; but we cannot exclude the possibility of deficient absorption of proteins (constant achylia, diarrhoea, etc.) in this disease as well, as there is no evidence in the literature of any investigations carried out on these lines. PETERS, EISENMAN and BULGER (1925) found low proteins in three out of seven cases of profound anaemia; in all moderate oedema was present, but no mention of the mechanism of the hypoproteinaemia in these cases was made.

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THERAPEUTIC INVESTIGATIONS.

1. In three cases the oedema disappeared within 2 to 4 days after admission on protein-free diet, suggesting that rest was the main factor in its cure and that circulatory fatigue is an important factor in its production.

2. In sixteen cases the oedema disappeared on protein diet within 10 to 15 days after admission before anything had been done to combat either the anaemia or the parasitic infections.

3. In three cases without changes in the blood chemistry, the oedema did not disappear until the anaemic condition improved.

4. Administration of marmite alone did not produce any improvement in ten cases, including three with and four without hypoproteinaemia.

CONCLUSIONS.

Twenty-two cases of helminthic anaemia with oedema were subjected to critical clinical, biochemical and therapeutic studies in order to investigate the various causative factors and the mechanism of production of the oedema. The following results have been obtained :—

1. Three cases showed a circulatory factor.

2. Three cases were due to the anaemic state.

3. Twelve cases showed hypoproteinaemia (biochemically investigated); four additional cases responded to high protein diet.

4. Neither the hypoproteinaemic nor the normal cases showed any evidence of avitaminosis B_1.

5. In addition to the demonstration of the various causative factors that may be concerned in the production of this oedema and the blood changes associated with it, the investigation indicates new lines of treatment for this condition. By replacing the deficiencies in the blood chemistry the equilibrium will be re-established, and not only the disappearance of the oedema but also adequate blood regeneration will be helped.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

THE RELATION OF BLOOD FEEDS TO THE MATURATION OF OVA IN *ANOPHELES ELUTUS.*

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INTRODUCTION.

Previous experiments (MER, 1936) showed that after the ovaries of *A. elutus* reached stage II in their development, a blood feed was essential for the maturation of the ova. Certain races of *Culex pipiens* are known to mature their eggs if fed only on sugar, raisins, etc.; in the Anophelinae, however, ingestion of blood seems to be a *sine qua non* for the maturing of eggs. In the experiments reported below an attempt was made to determine which fraction of blood contained the factor or factors indispensable for egg maturing in anopheles. Newly-hatched *A. elutus* females were used. These mosquitoes generally require two blood meals to bring the eggs to maturity. Feeding on sugar or fruit juices results in the development of the ovary only up to stage II (CHRISTOPHERS *et al.*, 1936) and then a single blood meal induces egg maturation.

Technique.

Freshly hatched *A. elutus* females were fed on whole blood, suspensions of washed red blood corpuscles in saline, serum, haemoglobin solutions in distilled water, sugar or raisins. After the digestion of the meal, the mosquitoes were dissected, the ovaries examined and their stage of evolution noted according to the scheme suggested by CHRISTOPHERS. Full blood meals were given by allowing the mosquitoes to feed on a donkey. Moist raisins and sugar solutions were introduced into the cages in which the mosquitoes were kept. Solutions of haemoglobin, suspensions of blood cells and serum were fed through a special animal membrane, in order to prevent their penetration into the diverticulum of the mosquitoes, which occurs regularly when the anophelines do not feed on an animal. In each experiment a number of mosquitoes were dissected to make sure that the food actually penetrated

into the midgut. In some experiments the exudate of cantharid blisters was used instead of serum.

EXPERIMENTS.

Experiment A. Feeding on Red Blood Cells. (Table I.)

The red blood cells of a donkey were separated from the serum by centrifugation and washing in saline three times. After the last washing a quantity of saline approximately equal to that of the plasma was added. The suspended cells were fed to anophelines through an animal membrane. The results are shown in Table I.

TABLE I.

EFFECT OF RED BLOOD CELLS ON MATURATION OF OVA.

	Number of Newly-hatched Female *A. elutus.*	Character of Feed.	Dissected after Digestion of 1st Feed.		Dissected after Digestion of 2nd Feed.	
			Number with Ovary Stage II.	Number with Ovary Stage V.	Number with Ovary Stage II.	Number with Ovary Stage V.
Experiment	57	Red blood cells suspended in saline	26	2	2	27
Control	57	Donkey	26	—	—	31

Experiment B. Feeding on Serum. (Table II.)

This series included three types of experiments: B, B_1, B_2. In Series B. serum separated from the coagulum and free from red blood corpuscles was fed to mosquitoes through an animal membrane. In Series B_1 gelatin in proportions of 1 : 3, 1 : 6, 1 : 10 was added to the same serum in order to make the mosquitoes retain the serum for a longer period in the midgut (pure serum disappears from the midgut during the first 36 hours after feeding, while gelatinized serum is digested as slowly as whole blood). In Series B_2 mosquitoes were fed on cantharid blisters; the fluid from these contains a serous exudate, a considerable number of white blood cells and a few red cells. The results of these experiments are summarized in Table II.

TABLE II.

EFFECT OF SERUM OR SEROUS EXUDATE ON MATURATION OF OVA.

Number of Newly-hatched Female *A. elutus.*	Character of Feed.	Dissected after Digestion of 1st Feed.	Dissected after Digestion of 2nd Feed.	Dissected after Digestion of 3rd Feed.	Dissected after Digestion of 4th Feed.
		Number with Ovary Stage I.	Number with Ovary Stage I.	Number with Ovary Stage I.	Number with Ovary Stage I.
Series B 36	Pure serum	10	3	2	11
Series B_1 36	Gelatinized serum	36	—	—	—
Series B_2 21	Blister exudate	5	16	—	—

Experiment C. Feeding on Haemoglobin. (Table III.)

Solutions of haemoglobin were prepared from donkey red blood cells separated from serum by centrifugation. The cells were laked by freezing and thawing or else distilled water was added to give a 50 per cent. solution. In either case the stroma of the red cells was removed by centrifugation. The haemoglobin solutions were fed to anophelines through an animal membrane. The results are summarized in Table III.

TABLE III.

EFFECT OF HAEMOGLOBIN ON MATURATION OF OVA.

Number of *A. elutus.*	Character of Feed.	Dissected after Digestion of 1st Feed.		Dissected after Digestion of 2nd Feed.		Dissected after Digestion of 3rd Feed.	
		Number with Ovary Stage I.	Number with Ovary Stage II.	Number with Ovary Stage I.	Number with Ovary Stage II.	Number with Ovary Stage I.	Number with Ovary Stage II.
42	Pure haemoglobin	4	15	—	5	1	17
23	50 per cent. solution of haemoglobin	4	4	3	12	—	—

It will be noted from the preceding experiments (Experiments B and C) that no maturing of eggs could be obtained either with serum alone or with haemoglobin alone. However, while the feeding on serum has no effect on the initial development of the follicles, feeding on haemoglobin leads to the development of the ova up to stage II. The effect of feeding on haemoglobin seems to be the same as that of feeding on sugar or raisins.

Experiment D. (Table IV.)

Since the ovaries of anopheles which had fed on raisins or sugar reach stage II and require very little blood for egg maturing it was to be expected that the specific egg maturing factor, contained in the blood, would be more readily detected if the various fractions were fed to insects previously maintained on these substances. In the following experiments serum or solutions of haemoglobin were fed to anopheles females which had been kept 6 to 8 days on raisins. The ovaries of these mosquitoes dissected before the serum or haemoglobin feed were always in stage II and the mosquitoes contained a certain amount of fat. The results of these experiments are shown in Table IV.

TABLE IV.

EFFECT OF HAEMOGLOBIN SOLUTIONS OR SERUM FED TO *A. elutus* FEMALES WHICH HAD PREVIOUSLY BEEN KEPT ON SUGAR OR RAISINS.

Series of *A. elutus* Females.	Character of Feed.	Dissected after Digestion of 1st Feed.		Dissected after Digestion of 2nd Feed.	
		Number with Ovary Stage II.	Number with Ovary Stage V.	Number with Ovary Stage II.	Number with Ovary Stage V.
D_1	Haemoglobin	39	—	14	—
D_2	Cantharid blister exudate	4	26	—	—
D_3	Serum	2	34	—	—
Control	Full blood	3	15	—	—

It is apparent from Table IV that haemoglobin solutions have no effect on the ovaries of anophelines in which the initial development of the follicles was completed up to stage II by a previous feed on sugar or raisins. On the other hand, under the same conditions, one serum meal leads to egg maturation. In this respect, the effect of the serum is equivalent to that of whole blood.

Experiment E. (Table V.)

From the results of Experiment C, it was to be expected that a feed on concentrated haemoglobin solution followed by a feed on serum would be sufficient for egg maturation. This proved to be the case, as is shown by the results tabulated below :—

TABLE V.

	Five Mosquitoes dissected after Digestion of Haemoglobin.		Thirteen Mosquitoes dissected after Digestion of Serum.	
Eighteen newly-hatched *A. elutus* females were fed once with haemoglobin and then with serum	Number with Ovary Stage II.	Number with Ovary Stage V.	Number with Ovary Stage II.	Number with Ovary Stage V.
	5	—	1	12

It appears, therefore, that the reason why blood is an essential food for egg maturation lies not in the need of haemoglobin but of some factor present in the serum. The haemoglobin can perform the same function as raisins or sugar and bring the evolution of the follicles up to stage II. Its effect is not, however, specific; the specific factor in the blood necessary for egg maturation exists in the stroma of the red cells and in cell-free serum.

NATURE OF EGG-MATURING FACTOR.

It was pointed out by MER (1936) that in *Anopheles* the process of development of the ovary may be divided into two distinct stages: (*a*) up to stage II and (*b*) from this to maturity. This division is not an artificial one; there is a basic physiological difference in the process of development. While the development up to stage II does not depend on blood or any of its constituents, the further development of the ovary is impossible without a factor which is apparently contained in serum as well as in the stroma of the red blood cells. In the following experiments we have attempted to ascertain the nature of this factor.

Experiment F. Effect of Heat. (Table VI.)

Whole serum was heated at 60° C. for 1 hour, and serum diluted in water 1 in 3 was heated to 100° C. for half an hour. The heated sera were then fed to mosquitoes, with ovary development at stage II, through a membrane.

The results of typical experiments are given in Tables VI and VII respectively.

TABLE VI.

HEAT RESISTANCE OF EGG-MATURING FACTOR IN SERUM.

	Ten dissected *before* Serum Feed.		Twenty dissected *after* Serum Feed.	
	Number with Ovary Stage II.	Number with Ovary Stage V.	Number with Ovary Stage II.	Number with Ovary Stage V.
Thirty *A. elutus* females were fed on raisins for 4 days, ten were then dissected; and the remaining twenty were fed on serum which had been heated at 60° C. for 1 hour	10	—	1	19
Control— Twenty-one *A. elutus* females were fed on raisins for 4 days; eight were then dissected and the remaining thirteen were fed on unheated serum	Eight: 8	—	Thirteen: 2	11

TABLE VII.

HEAT RESISTANCE OF EGG-MATURING FACTOR IN SERUM.

	Number of *A. elutus* Females with Ovaries at Stage II.	Character of Feed.	Dissected after Serum was Digested.	
			Number with Ovary Stage II.	Number with Ovary Stage V.
Heated serum.	23	Serum (1 : 3) heated for 30 minutes at 100° C.	18	5 (21 per cent.)
Control, with unheated sera.	*a.* 50	Whole serum	4	46 (92 per cent.)
	b. 32	Serum diluted 1 : 1	17	15 (46 per cent.)
	c. 28	Serum diluted 1 : 2	20	8 (28 per cent.)
	d. 18	Serum diluted 1 : 3	14	4 (22 per cent.)

These experiments show (1) that the egg-maturing factor is heat-resistant and (2) that a certain concentration (or quantity) is required. Dilution of the serum, heated or unheated, decreases the percentage of mosquitoes with mature ovaries.

Experiment G. (Table VIII.)

In this experiment, the serum was heated to 100° C. for 15 to 20 minutes and the coagulated proteins separated by centrifugation. The supernatant fluid was decanted and the sediment dried. After drying, the powdered proteins were resuspended in water in a dilution of 1 in 4; 5 per cent. gelatin was added in order to obtain a stable suspension.

Mosquitoes of the same batch were then fed through a membrane either with the supernatant fluid or with the serum suspension. The results are given in Table VIII.

TABLE VIII.

PRESENCE OF EGG-MATURING FACTOR IN THE SERUM PROTEINS OR SERUM WHEY.

Number of Anopheles Dissected after Digestion of Feed.	Character of Feed.	Number with Ovary Stage II.	Number with Ovary Stage V.
13	Serum whey	13	—
30	Serum protein suspension in water (1 : 4)	5	25 (82·5 per cent.)

It is apparent from the data given in Table VIII that the egg-maturing factor is associated with the proteins of the serum and that its efficacy is not reduced by heating to 100° C. and subsequent drying.

Experiment H. Function Lipoids. (Table IX.)

In this experiment serum was treated with ether, allowed to stand for 24 hours with repeated shaking. The ether layer was then removed, evaporated, and the ether-free residue emulsified in a concentrated solution of haemoglobin.

Anopheles of the same lot, with ovaries in stage II of their development. were fed on the lipoid-free serum and on the lipoid suspension respectively, The results are given in Table IX.

TABLE IX.

EFFECT OF LIPOIDS, EXTRACTED FROM SERUM OR FROM RED CELLS, ON MATURING OF EGGS.

Number of Anopheles.	Character of Feed.	Dissected after Digestion of Meal.
(*a*) 15	Serum after extraction of lipoids	All (15) had ovary Stage V
(*b*) 14	Serum lipoid suspension	All (14) had ovary Stage II
(*c*) 14	Red cell-lipoid suspension	All (14) had ovary Stage II

These results demonstrate that the lipoids have no effect on egg maturing; the lipoid-free serum, however, still retains its full potency in this respect.

SUMMARY.

A study was made of the blood elements essential for the maturation of eggs of anopheles mosquitoes. The results may be briefly summarized as follows :—

1. Blood contains a factor which is necessary for the maturation of eggs of *A. elutus*.

2. Neither haemoglobin alone nor serum alone is capable of inducing egg maturation.

3. Haemoglobin can bring the ovaries to stage II of their development but no further ; in this respect it serves as do raisins and sugar.

4. If anopheles whose ovaries are at stage II are fed on serum, then complete ripening of the ovaries occurs.

5. The specific egg-maturing factor is found, therefore, in the stroma of the erythrocytes as well as in the cell-free serum.

6. This factor is still active after the serum has been heated at 100° C. for 1 hour.

7. In serum coagulated by boiling the specific factor is associated with the coagulum and is absent in supernatant fluid.

8. Lipoids extracted from serum or red cells are inactive, whereas the extracted serum is still active.

9. It is not clear whether we are dealing with an accessory substance or with the specific action of the protein itself. It is clear that the substance is heat-stable, and is associated intimately with the serum protein and cell stroma. It appears also that a certain quantitative relation exists because, when the serum is diluted progressively, fewer and fewer of the anopheles fed mature their ovaries.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

STUDIES ON THE GABOON VIPER (*BITIS GABONICA*) AND THE PREPARATION OF A SPECIFIC THERAPEUTIC ANTIVENENE.

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Of the interesting results afforded by continuous researches on the polyvalent action of antivenenes prepared in our Serum Department, probably the most significant are those connected with the venom of the Gaboon viper (*Bitis gabonica*). We have shown (GRASSET and ZOUTENDYK, 1936 and 1937) that the concentrated polyvalent antivenene prepared by us from puff adder (*Bitis arietans*) and Cape cobra (*Naia flava*) anavenoms exhibits but a negligible neutralizing action against the venom of the Gaboon viper ; and, with the exception of some sera of Asiatic origin, this lack of action is even more marked in the case of those numerous sera of European, South American and South African origin which we have had the opportunity of testing in the course of very extensive comparative investigations. One can, therefore, conclude that no serum has hitherto been available for the treatment of bites by the largest viper in Africa, and although this is of no practical significance in the Union since the species does not occur here, it is a distinct hazard in those equatorial regions of Africa where it is comparatively common. Conversation and correspondence with well informed persons who have spent many years in tropical Africa tend to show that considerable confusion exists regarding the identification of the Gaboon viper—due to its nocturnal habits, its lethargic nature and its natural protective colouring. It is hoped that the following

description (from the *British Museum Catalogue of Snakes*, iii, 499) and the plates at the end of this paper will assist in clearing up this difficulty to some extent.

Bitis gabonica.

" Nostrils directed upwards and outwards. Rostral very small, once and a half to twice and a half as broad as deep ; head covered with small, moderately keeled scales, smallest on the vertex, 13 to 16 from eye to eye ; 15 to 19 scales around the eye ; four or five series of scales between the eye and the labials ; a pair of more or less developed, compressed, erectile, triangular, sometimes bi- or tri-cuspid shields in contact with each other, between the supranasals, forming a pair of nasal horns ; four or five series of scales between the nasal and the rostral ; 13 to 16 upper labials ; four or five lower labials in contact with the chin shields. Scales in 33-41 rows, strongly keeled, outer row smooth ; lateral scales slightly oblique, pointing downwards. Ventrals 125-140 ; anal entire ; subcaudals 17-33. Brown above with a vertebral series of elongate, quadrangular, yellowish or light brown spots connected by hour-glass shaped dark brown markings , a series of crescentic or angular dark brown markings on each side ; head pale above, with a dark brown median line, a dark brown oblique band behind the eye, widening towards the mouth ; yellowish beneath with small brown or blackish spots.

Total length 1,170 millimetres ; tail 70 millimetres.

Tropical Africa (West Africa from Liberia to Damaraland ; Zanzibar ; Mozambique)."

In a recent comprehensive study of the reptiles of French West Africa (ANGEL, 1932), the length of a specimen is given as 1,750 mm. The author also describes and illustrates *Bitis nasicornis* which we have reason to believe is frequently confused with the Gaboon viper even by people who have spent many years in the tropics. This mistake is hardly likely to occur if it is remembered that the Gaboon viper possesses a pair of comparatively insignificant horns set closely together whilst *B. nasicornis* possesses two or three pairs of much more prominent hornlike shields separated by small scales ; the colour of the latter snake is purple or reddish brown with olive or dark brown spots, a vertebral series of brown spots and an olive belly spotted with black or yellow. Its venom when desiccated is white compared with the canary yellow colour of Gaboon viper venom.

GABOON VIPER VENOM.

We have not been able to trace any researches on the venom of the Gaboon viper, it having been apparently overlooked by workers in the field of immunological studies of venoms and antivenenes.

Yield.—The average yield may be taken as 200 mg. venom when desiccated ; but individual specimens have yielded as much as 300 mg. and in one instance 484 mg. were obtained. By manipulating the glands of a dead Gaboon viper which measured 1,150 mm. in length, a volume of 1·73 c.c. was obtained from one gland.

Toxicity.—The toxicity of *Bitis gabonica* venom as compared with that of *B. arietans* is shown in the following table ; in every case the fatal dose kills in 12 to 24 hours.

TABLE I.

TOXICITY OF THE VENOM OF *Bitis gabonica* COMPARED WITH *Bitis arietans* VENOM.

	Route.	Venom in mg.	
		Bitis gabonica.	*Bitis arietans.*
Mouse	Subcutaneous	0·4	0·15
Rat...	„	3	1·5
Guineapig (500 grammes) ...	„	75	50
Rabbit (1,500 grammes) ...	Intravenous	3·2	1
Pigeon	„	0·075	0·075*
	Subcutaneous	2·5	2
Fowl	Intravenous	0·6	1·5
Sheep	Subcutaneous	80	50

**Note.*—In a paper dealing with the toxic and antigenic properties of snake venoms (GRASSET, ZOUTENDYK and SCHAAFSMA, 1935), the fatal dose of *Bitis arietans* venom for the pigeon appeared as 0·75 mg.; this should read 0·075 mg. as in the present table.

The figures indicate that the potency of *Bitis gabonica* is considerably lower than that of *B. arietans* when administered subcutaneously; but, except in the case of the rabbit, this difference is more than compensated for by the highly coagulant nature of *B. gabonica* venom when administered intravenously; this coagulant property has been the subject of considerable investigation in connexion with haemophilia in humans.

DETOXICATION OF *Bitis gabonica* VENOM AND INVESTIGATION OF ITS ANTIGENIC PROPERTIES.

By subjecting 1 per cent. solutions of *B. gabonica* venom to the action of 0·75 per cent. formalin at incubator temperature, we were able to procure atoxic derivatives analogous to those originally obtained by us in the case of *B. arietans* and other viperine and colubrine venoms. By dissolving the venom in normal saline the average time of incubation necessary for detoxication was from 5 to 6 weeks, but this period could be reduced considerably by the substitution of Martin's broth for saline, the aspecific protein playing a similar role here to that which we have already reported upon in connexion with the detoxication of various colubrine venoms. Rabbits injected intravenously with 50 mg. of the detoxicated material survived without ill effects. That the antigenic properties remained unimpaired was demonstrated by the subcutaneous immunization of rabbits with the anavenom; animals which received three injections of 30 mg., 50 mg. and 70 mg. at intervals of 8 days were so highly protected that they were able to withstand intravenous test doses of 15 mg. *B. gabonica* venom, controls dying regularly in from 10 to 17 minutes. The possession of this atoxic antigenic material

facilitated the carrying out of comparative tests with *B. arietans* anavenom on the following lines :—

Two groups of rabbits were taken ; Group I received three subcutaneous injections of 30 mg., 50 mg. and 70 mg. *B. gabonica* anavenom at intervals of 8 days, Group 2 receiving the same quantities of *B. arietans* anavenom. On being tested intravenously at the end of this course of immunization the animals reacted as follows to specific and cross neutralization tests.

TABLE II.

THE SPECIFIC AND CROSS PROTECTION AFFORDED BY *Bitis gabonica* AND *Bitis arietans* ANAVENOMS.

	Limit of Specific Protection.	Limit of Cross Protection.
Rabbits immunized with *Bitis gabonica* anavenom	15 mg. *B. gabonica* venom (5 fatal doses)	10 mg. *B. arietans* venom (10 fatal doses)
Rabbits immunized with *Bitis arietans* anavenom	10 mg. *B. arietans* venom (10 fatal doses)	No protection against *B. gabonica* venom

The entirely unexpected fact is thus revealed that of two species zoologically classified as belonging to the genus *Bitis*, the venom of one (*Bitis gabonica*) possesses an additional fraction, probably coagulant, which is not included in the antigenic structure of the venom of *Bitis arietans*. The importance of this fraction is emphasized by the fact, already alluded to, that no antivenene, whether of African or overseas origin, among all those with which we have had the opportunity of working, is capable of exerting any appreciable action against the venom of the Gaboon viper ; the preparation of an antivenene possessing specific neutralizing properties is, therefore, highly desirable.

PREPARATION OF A SPECIFIC *Bitis gabonica* ANTIVENENE.

The chief difficulty in applying the knowledge already gained to the hyperimmunization of horses was the question of adequate supplies of Gaboon viper venom ; thanks, however, to the co-operation of Mr. B. PEERS of the Cape Town Snake Park, and of a few public health authorities in tropical Africa, we were furnished with approximately 3½ grammes of the venom ; and we had perforce to rest content with this, for when it became known that no available serum was of therapeutic value in the event of a bite there was an understandable reluctance to handle living specimens of a formidable reptile like the Gaboon viper.

With the exception of a small quantity retained for titration purposes, the available supply was converted into Gaboon viper anavenom by submitting

a 1 per cent. solution in Martin's broth to the action of 0·75 per cent. formalin at 37° C. for 3 weeks. At the end of this period Horse 278 received a preliminary subcutaneous injection of 50 mg. and continued to receive weekly injections consisting of, respectively, 100 mg., 200 mg., 300 mg., 400 mg., 500 mg., 600 mg., 700 mg., and a final injection of only 400 mg. due to the exhaustion of the supply of antigen. The neutralizing properties of the serum were assessed at different stages of the immunization, the tests being carried out by the intravenous injection of rabbits weighing 2,000 grammes with mixtures of serum and venom which had remained in contact at laboratory temperature for 1 hour. The samples of blood were taken from the horse immediately prior to an injection, i.e., 7 days after the preceding dose. The tests evinced a progressively increasing neutralizing action. For example, after the fourth injection (300 mg.) the action was negligible and irregular, whilst after the fifth (400 mg.) the time of death was delayed to 16 hours in rabbits injected with mixtures consisting of 3 c.c. serum and 7·5 mg. venom. After seventh injection (600 mg.) rabbits receiving similar test doses survived without ill effects, as did those receiving mixtures consisting of 2 c.c. serum and 5 mg. venom. A bulk bleeding was carried out 10 days after the last injection, tests showing that 3 c.c. serum were sufficient to neutralize 10 mg. venom, 1 c.c. being thus able to neutralize approximately one fatal dose for the rabbit. Cross neutralization tests showed that 3 c.c. of the serum were able to neutralize 5 mg. puff adder venom (five fatal doses for the rabbit), this confirming the results already referred to in which rabbits were submitted to active immunization with the respective anavenoms.

Although the neutralizing power of the serum of Horse 278 was sufficiently high for our immediate purpose, there is no doubt that a larger supply of antigen would have afforded even more gratifying results by making possible a more intensive and extended course of immunization.

CONCENTRATION OF THE *Bitis gabonica* ANTISERUM.

All antivenene produced in our Serum Department is concentrated and refined by fractional precipitation with sodium sulphate; the newly-produced Gaboon viper antiserum was submitted to the same process with equally successful results. Tests carried out with the resulting pseudoglobulin showed that 0·45 c.c. was able to neutralize 5 mg. venom, the concentrated and refined serum being thus 3·3 times more potent than the natural serum from which it was derived. After diluting the pseudoglobulin with distilled water for therapeutic use, 1 c.c. of the finished product neutralized 7 mg. Gaboon viper venom; since a dose of 20 c.c. is recommended for the treatment of a bite by the average sized specimen, a total quantity of 140 mg. venom would be neutralized, this being approximately equivalent to the amount delivered by a normal bite if one can judge by the weight of venom obtained by manipulation of the glands. It is therefore possible to treat bites by the Gaboon viper with

a specific antivenene and there is consequently a real need for either the production of a monovalent serum or the incorporation of Gaboon viper anavenom in the antigen employed for the preparation of a polyvalent antivenene destined for use in equatorial Africa. Lack of the necessary venom is the only obstacle; and now that specific protection is available for workers in this field it is hoped that with the aid of health officials in the territories concerned this difficulty will be overcome in the near future.

SUMMARY.

The *Bitis gabonica* (Gaboon viper) is described and contrasted with the *Bitis arietans* (puff adder) and the *Bitis nasicornis*.

Emphasis is laid on the fact that no domestic or foreign serum hitherto tested has exerted more than a negligible neutralizing action on *B. gabonica* venom.

B. gabonica venom is characterized by a toxic fraction not present in *B. arietans* venom; rabbits immunized with anavenom derived from the former show a high resistance to *B. arietans* venom, whilst injections of *B. arietans* anavenom afford no protection against *B. gabonica* venom.

B. gabonica venom may be rendered atoxic by means of formalin; the resulting anavenom is antigenic and gives rise in the horse to a specific antivenene of therapeutic value.

The natural serum may be concentrated and refined by means of sodium sulphate, the neutralizing power being increased more than threefold.

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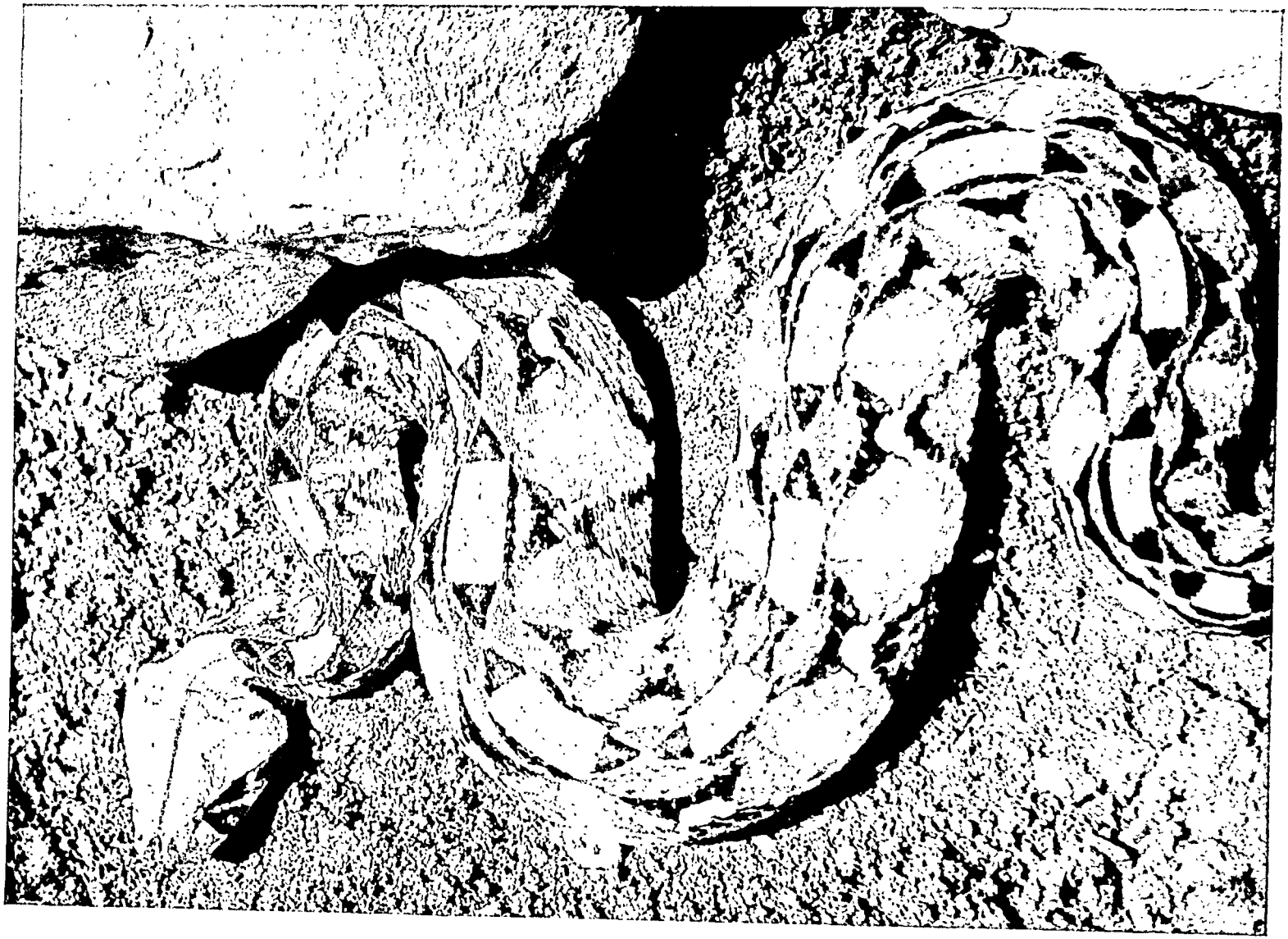


FIG. 1.—*Bitis gabonica* under natural conditions.

Photographs 1 *and* 2 *by Mr. B. Peers, Cape Town.* *Photographs* 3 *to* 8 *by Mr. F. Brandt, South African Institute for Medical Research, Johannesburg.*



FIG. 2.—*Bitis gabonica* under natural conditions.

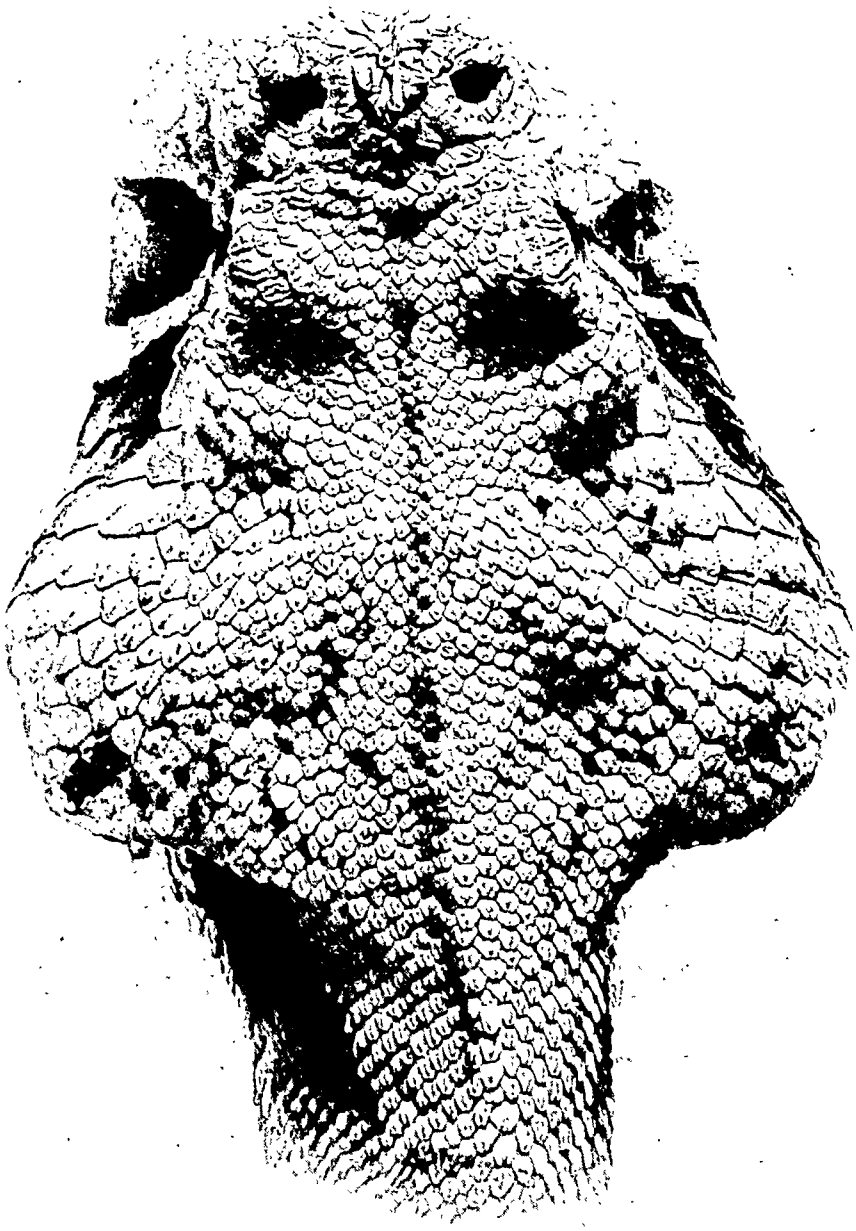


FIG. 3.—Head of *Bitis gabonica*. Dorsal view.

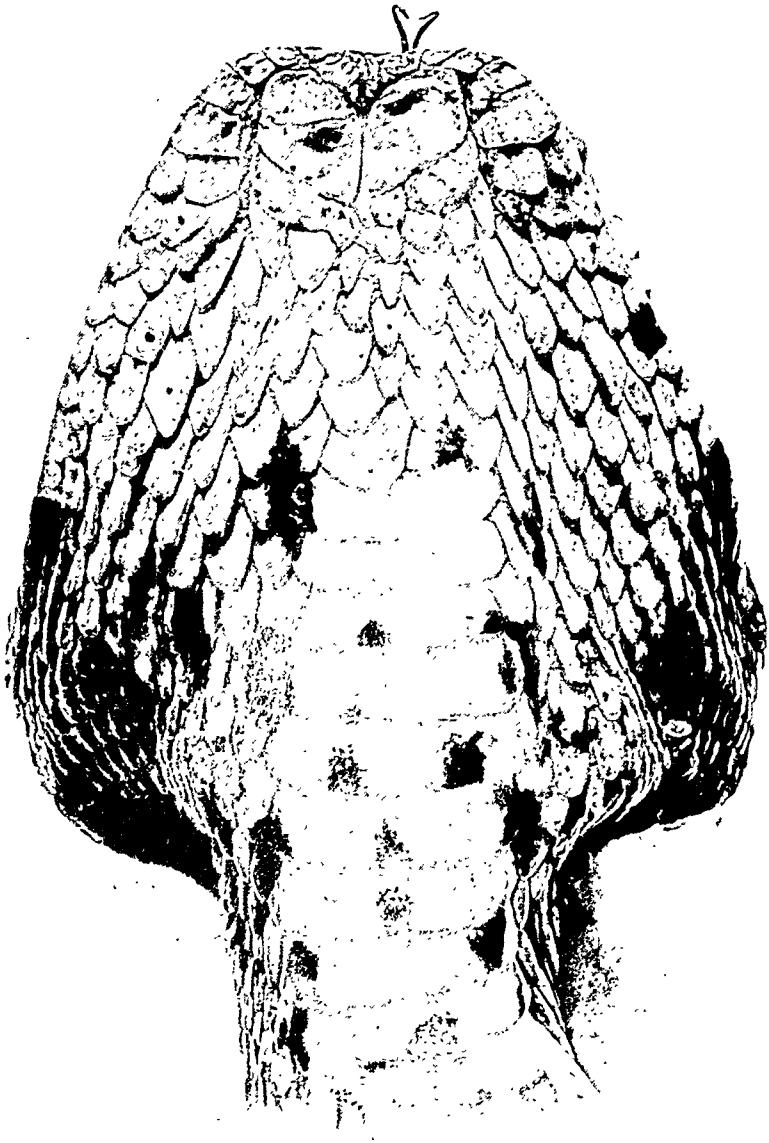


FIG. 4.—Head of *Bitis gabonica*. Ventral view.



FIG. 5.—Head of *Bitis gabonica*. Side view.



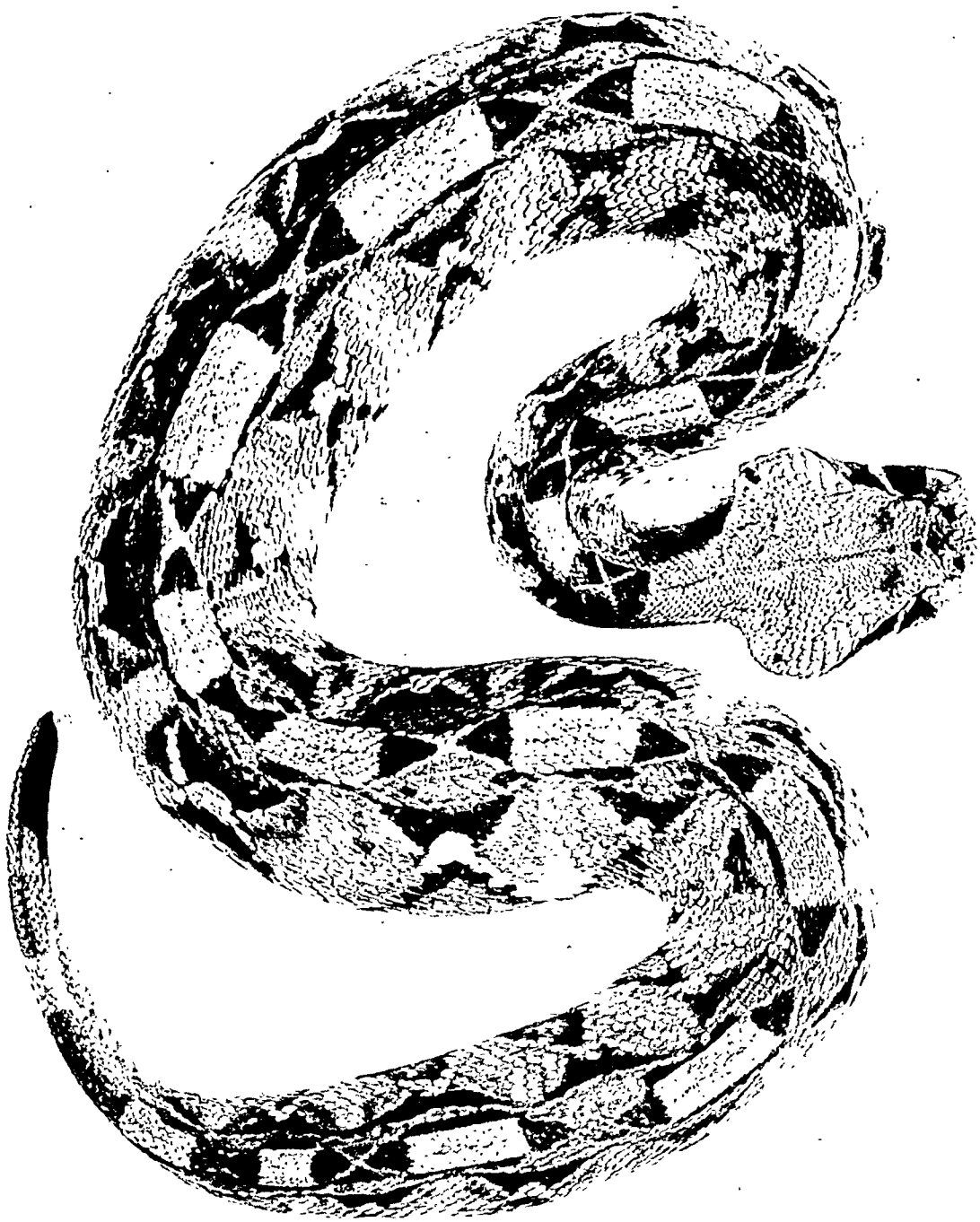


FIG. 7.—*Bitis gabonica* showing hour-glass markings.

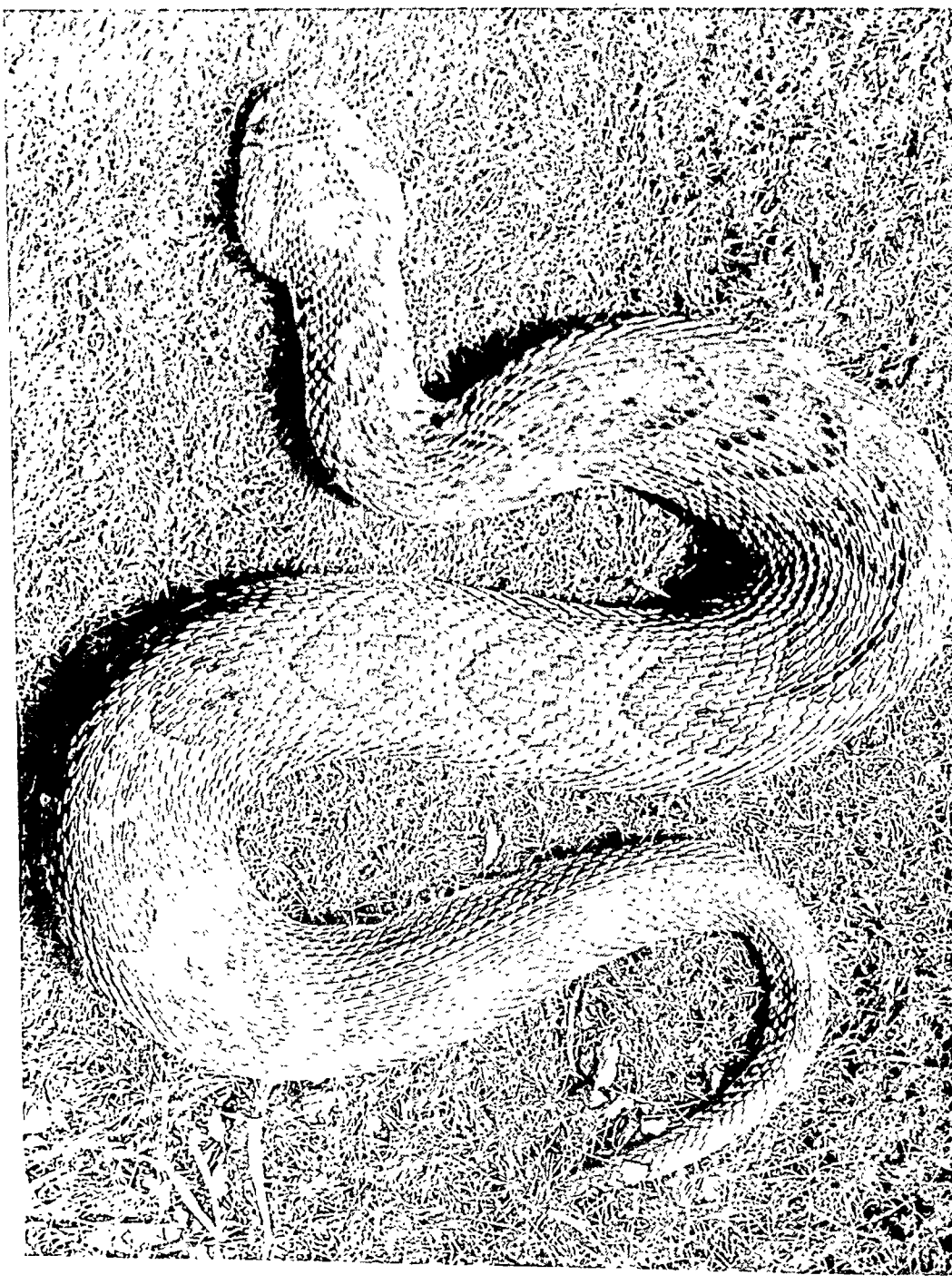


FIG. 8.—*Bitis arietans* under natural conditions.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

WARMTH AND HUMIDITY AS PREDISPOSING FACTORS IN THE INCIDENCE OF YAWS.

BY

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The purpose of this article is to emphasize the fact that the chief factors in the distribution of yaws are a warm climate and humidity. Factors such as rainfall, geological formation of soil, vegetation, altitude, distance from the sea are of importance only in so far as they affect humidity. Any influence which unhygienic conditions may exert or which flies, such as *Hippalates pallipes*, may have in the dissemination of the disease can occur only in areas with a tropical, humid climate.

These deductions have been made as a result of the study of environmental factors and of their comparison in areas of Central America and of the West Indies where there is a high incidence of yaws with areas in the territories named in which there is no yaws. These investigations began on a visit to Central America and to Cuba in September, 1933, and were continued in Jamaica from October, 1933. The world distribution of the disease as expressed in the literature on yaws tends to confirm these deductions.

The disease is rarely found in countries where the mean isotherm is under 70° F. In countries lying between isotherms 70° F. and 79° F. the incidence of the disease is moderate except in regions with very heavy rainfall. In regions with isotherms of 80° F. and over the disease abounds, provided these regions are not arid or semi-arid.

When these general principles are considered in relation to Jamaica, a small island surrounded by the Carribbean Sea, it is found that the distribution of yaws is very variable. The island records of the averages of minimum and maximum temperature of places where there is yaws and where there is no yaws, do not, however, reveal any parallel differences that could account for this uneven distribution of the disease.

The living conditions and the pursuits of the masses throughout the island are fairly constant. Neither also from hygienic nor from economic standpoints is there any accountable difference between many areas where yaws abounds and those where there is no yaws. Unhygienic and poor economic conditions play important roles in the spread of the disease, but they are not in themselves essential factors, for in the slum areas of large towns in the island yaws does not occur. The occasionally reported cases have always been brought in from rural areas and give no cause for anxiety to the sanitary authorities: the disease quickly dies out again. Yet, hygenic and economic conditions in the slums show no improvement on those of rural yaws-infested areas.

It has been found that in Jamaica where there is yaws the rainfall is generally fairly high, but the reverse is not always true. There are areas with little or no yaws which have a heavier annual rainfall than many yaws-infested areas. For example, in two rural districts, Duncans and Devon, widely separated, there is no yaws, yet the average annual rainfall for the past 60 years is 80 inches 41 parts, and 73 inches, respectively. Duncans is 300 feet above sea level and Devon 2,560 feet. In another district, Duanvale, of elevation approximately 500 feet, and with an average annual rainfall for the past 60 years of 51 inches, the incidence of yaws is fairly high. Duanvale is only about 7 miles from Duncans. In a rural district, Crawle, which lies between Duanvale and Duncans, with an approximate elevation of 400 feet, there is no yaws among its population of over one thousand. Several like instances could be stated of areas in Jamaica where there is a heavy annual rainfall but with little or no yaws. There are also districts situated 1,800 feet to 2,300 feet above sea level where over 57 per cent. of the population give a history of yaws, while in other districts of an equal elevation yaws does not exist.

A study was made of the differences in living conditions and of environmental factors between Duncans and Crawle on the one hand and Duanvale on the other hand. The most striking difference was that while Duncans and Crawle in spite of heavier rainfall appeared to be well drained and comparatively dry, Duanvale was to all appearances damp. The presence of a swamp and of a few ponds in Duanvale emphasized this fact. The underlying geological

formation of these districts is limestone. To the south of Duanvale is a range of hills on a geological formation of conglomerates. The seepage of water from these hills carries in solution deposits which on settling make the top soil of Duanvale a marly alluvium. This surface soil is more tenacious than the top soil of Duncans and Crawle which are further removed from the hills. Because of the underlying limestone formation there are no rivers to carry deposits from the foothills to the plains on which the districts of Crawle and Duncans lie. For a time, however, it was thought that a certain pH of the soil may have been the cause which determined the presence or absence of yaws in a district. It was found that in some districts there is a distinctly alkaline soil with a high incidence of yaws. This also holds good in districts where there is no yaws. The only characteristic constant and predominant in all areas with a high incidence of yaws is a higher state of humidity as compared with areas with little or no yaws. This higher degree of humidity may be gauged by the amount of moisture in the surface soil. The incidence of yaws tends to rise in so far as there are factors which tend to increase the dampness of the soil. This dampness in turn is dependent on the colloidal content and the associated capillarity of the soil or on its imperviability, or on other conditions encouraging dampness, such as poor drainage, seepage from watersheds, a persistently high sub-soil water level, or limitations to evaporation such as may occur in sun-starved valleys. The greatest contributory factor lies in the colloidal content and in the thickness of the top soil. When the surface soil is loose, water sinks rapidly till an impervious sub-soil is reached. The tendency is for the surface soil to be kept moist by water that rises from this sub-soil. As a result of the colloidal content of heavier soils there will be a greater tendency also to deposits of hygroscopic water. Coarse, sandy soils obviously cannot hold water as do clay soils. STOCKLEY* states that the colloid content of calcareous soils differs from normal clay or from soil of igneous origin ; that under drought conditions calcareous soils become gradually dehydrated at a quicker rate than do normal clay soils or soils derived from igneous rocks, both of which hold water more effectively. Thus, the non-moisture-retaining qualities of sandy soils with the very partial retaining qualities of soils of limestone formation tend to reduce humidity, and this accounts for the absence of yaws in such areas. In areas where the surface soil is of marl or alluvium some yaws will be found if any of the factors encouraging dampness are present. There is marked correlation between the heavier geological formations in Jamaica—such as carbonaceous shale, yellow limestone, conglomerates and igneous rocks—with a high incidence of yaws.

Very little purpose would be served in giving a long list of districts with a high incidence of yaws and of the respective factors which make them damp, or of districts with little or no yaws and of the respective factors which tend to

*STOCKLEY, G. M. (1925). *Report of the Government Geological Department*, 27th March, p. 26. Jamaica : Government Printing Office.

lower the humidity. A few illustrations will suffice to emphasize the points mentioned.

There are a few places which have little or no yaws notwithstanding an impervious soil. There must be other factors to produce this incongruity. These may be improved drainage, the paving of roads and pathways as in some townships, a larger percentage of people wearing shoes and stockings, improved types of dwellings with advanced sanitation which necessarily reduce the evils of overcrowding.

To illustrate the relationship of humidity with a high incidence of yaws, a district came under purview in which the top soil was a sandy loam but which had other features that tended to render it damp. This district, although at an elevation of 600 feet, is surrounded by mountains rising to 1,730 feet towards the east and to 2,000 feet towards the west; these serve to cut off hours of sunshine in the morning and in the evening. The mountains lie on an impervious formation of igneous and conglomerate rocks. Seepage of water into the soil of this district is abundant. The standard of living is not below the average, yet the percentage of persons with a history of yaws was found to be 76·38, a percentage which is unusually high and only equalled in another district which lies in a valley and where the soil consists of impervious conglomerates and carbonaceous shale. The standard of living of the people in this district is somewhat below the average for rural Jamaica. Another district adjoining the first mentioned (with 76·38 per cent. incidence of yaws) is comparable to it in respect to soil, elevation, rainfall, standard of living and seepage from watersheds, but owing to the arrangement of the mountain ranges which run due east and west it receives more of the sun's rays and is therefore not as damp. The percentage of yaws is here 60·5.

It is hoped that in the near future records will be obtained of the relative humidity of several yaws endemic areas for comparison with areas where there is no yaws. At present meteorological records on humidity are only available for one of the areas in Jamaica with a high incidence of yaws. A comparison is made of the relative humidity of this area during 1 month in summer, 1 month in autumn and 1 month in winter, with another area 16 miles away where there is no yaws. The relative humidity of the yaws endemic area was consistently higher than that of the area without yaws. The mean relative humidity for the period of observation was 89·6 per cent. in the yaws endemic area as compared with 74·5 per cent. for the area with no yaws.

If humidity in relation to yaws incidence is of the importance that is claimed for it, then in the same district under more humid conditions of rainy seasons an increase in the prevalence of the disease is to be expected in comparison with drier seasons of the year. As a test a census was taken and examinations for yaws lesions carried out on all persons living in one small defined area after 2 months of moderate rains. The area was left untreated until the end of a dry season when as many of the same individuals as possible were re-examined.

The rainfall for the 2 months preceding the first census was 12 inches 63 parts, and that for 2 months preceding the re-examinations was 3 inches 73 parts. The number of persons with lesions in this area was definitely higher by 9·2 per cent. during the wet season than in the dry season. The higher percentage was due almost entirely to the larger number of individuals with infectious lesions after the wet season. The total number of persons examined at the end of the wet season was 625. Of these only 500 were available for a second examination at the end of a *dry* spell. (Forty-five having died or removed in the interval, and eighty more not being found.) Of these 500 cases (examined twice)

116 gave a history of a first attack of yaws 4 years ago
128 „ „ „ „ „ „ over 4 years ago.

This makes a total of 244 (48·8 per cent.) with a history of yaws and therefore possibly with some degree of immunity; while the remaining 256 (51·2 per cent.), giving no history of yaws, may be considered as possibly non-immune.

Lesions found on Examination.	Wet Season.		Dry Season.	
	Number.	Per cent.	Number.	Per cent.
Infectious lesions	60	12·0	15	3
Non-infectious lesions	36	7·2	35	7
Totals	96	19·2	50	10

A similar estimate was made in another area after two separate dry periods. There was no appreciable difference in the number of persons with lesions. Other estimates of the prevalence of the disease in the same area in wet seasons as compared with dry seasons showed an increase of lesions, mostly of the infectious type, in the wet seasons.

In damp or humid areas vegetation is usually profuse. Vegetation tends to increase humidity. Any other influence of vegetation in the spread of the disease is not upheld. The presence of increased numbers of flies such as *Hippalates pallipes* in yaws endemic areas seems incidental to factors promoting dampness and to the presence of food supplies for the flies, among which yaws sores would figure prominently. In many dry areas of Jamaica there is less vegetation but there are many briars which provide greater facilities for injuries to the extremities. One might think these injuries would provide means for dissemination of the disease, but this does not appear to be the case. There is no

reason to believe that any type of vegetation would sustain the infecting organism outside the human body.

In areas where there is little or no yaws, but which are readily accessible to persons from yaws endemic areas, a temporary increase or a few cases may occur in abnormal periods of humidity dependent on prolonged rainy seasons.

The true position seems to be that a humid atmosphere slows up the evaporation of moisture from the skin. A moist skin seems to have some influence on the exuberance of skin lesions. *Treponema pertenue* coming in contact with moist surfaces whether of skin or of soil, will survive for longer periods than it does with dry surfaces.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 4. January, 1938.

THE CHINESE URBAN DEATH RATE IN HONGKONG.

BY

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INTRODUCTION.

The geographical, social and climatic conditions of Hongkong are described in detail in a paper now in the press (UTTLEY, 1938) and only a brief outline will be given here.

Hongkong is just within the tropics on the coast of South China. It is subject to the effects of the monsoons, November to March being dry and cool, and the rest of the year being hot, damp and muggy, when most of the annual 87 inches of rain fall. Of the population 97 per cent. are Chinese, who live either in the two cities, Victoria, on the Island of Hongkong, and Kowloon, which is on the mainland across the harbour; in the country villages; or on the sampans and junks in the waters about the colony. Their main occupation is commerce and trade with the neighbouring parts of China, and a considerable minority of the population is constantly on the move to and from China. It is stated that 7,000 persons enter or leave the colony daily.

With such a large migration constantly taking place, any attempt to calculate a standardized death rate is bound to result only in an approximation. Nevertheless the attempt is justified since death registration in Hongkong is accurate, and the census returns are very fairly accurate, while no such calculation has hitherto been made either for Hongkong or for any Chinese city.

The results I have obtained, though not as accurate as similar data for Western European countries or North America, are I believe reasonably near the truth.

This paper discusses only the urban Chinese of the colony. While personally scrutinizing each of the 420,000 death returns dealt with in this paper, I excluded those occurring among the non-urban population, such as the country dwellers, harbour inhabitants, those passing through the colony and also those of non-Chinese. Similarly census figures concerning only the urban Chinese of Victoria and Kowloon were used, and not those for the whole colony. It is necessary to emphasize this, because all official returns dealing with disease and deaths in the colony relate to the colony as a whole, and up to the present no attempt has been made to consider one section of the population alone; from a statistical point of view such official data are of little value.

Victoria throughout the last 50 years has been very overcrowded, and is no less so to-day than in the past. Its 740 acres of built-over land house over 400,000 people. In Kowloon the greater proportion of its 250,000 inhabitants live in an area accommodating over 300 to the acre. Victoria and Kowloon are in many respects one city separated by a mile of harbour; in this paper they will be taken together as such when dealing with data referring to 1901 or later. Prior to that date, Kowloon was too small to be considered an urban area, and I have not included it in my figures for 1897 to 1900. Whenever the name Hongkong is used in this paper, it refers to the urban areas I have mentioned, and not to the colony as a whole.

The Census Populations.

Table I shows the growth and the structural changes which have taken place in the urban Chinese population of Hongkong since 1897. In that year the first census sufficiently accurate for the purpose was taken. All figures are adjusted to estimated mid-year populations in the census years.

In the early years of the colony, *i.e.*, in the 'forties and 'fifties of last century, Hongkong from the Chinese point of view was a place to which the young adult male came to seek a living; but from which, after the best years of his life were over, he departed to rejoin his wife and children who had been left behind in the ancestral village up country. This might lie anywhere in the neighbouring provinces of China. By the middle of the 'eighties some of the labouring classes began to bring their brides or families to Hongkong to live, after the

husbands had found an occupation. By the turn of the century this tendency was well marked, and has continued at an ever increasing rate. As conditions became more favourable for the growth of family life, and incidentally as the standard of life improved, this tendency to bring relatives and dependents to Hongkong became the rule: no longer were children, born in Hongkong, sent back in large numbers to the ancestral village to live until old enough to be married or to earn a living.

TABLE I.

HONGKONG URBAN CHINESE POPULATIONS AT SUCCEEDING CENSUSES, 1897-1931, WITH THE PERCENTAGE OF THE POPULATION IN EACH AGE GROUP.

Age in Years.	1897	1901	1911	1921	1931
0 upwards	4·42	3·94	4·70	7·78	8·59
5 ,,	5·37	4·81	6·15	7·03	7·44
10 ,,	6·27	6·16	6·84	8·85	8·14
15 ,,	25·53	27·55	24·30	25·33	25·21
25 ,,	25·71	26·92	26·06	22·43	21·21
35 ,,	17·05	16·58	17·82	13·81	14·57
45 ,,	10·33	9·07	8·93	8·05	8·84
55 ,,	4·13	3·84	3·77	3·28	4·33
65 ,,	0·98	0·90	1·15	1·23	1·31
75 ,,	0·19	0·20	0·27	0·20	0·40
85 ,,	0·01	0·04			
Totals—					
Males	117,544	163,169	195,322	272,153	371,906
Females	45,531	57,150	90,796	162,571	268,850
Persons	163,075	220,319	286,118	434,724	640,756
Intercensal increase		57,244	65,799	148,606	206,032

These changes show themselves in the figures of Table I, where it is seen that a steady increase has taken place in the relative and absolute numbers of the very young and the elderly during the last 40 years. There has always been a preponderance of males, though now much less than in the past.

There are two points to which attention should be drawn in these census figures. In those for 1911 there are stated to be included about 20,000 refugees who had fled from the revolution which resulted in the destruction of the Manchu dynasty. The census in 1921 was taken at a time of poor trade conditions when a certain unknown number of people would have returned to their homes up country to await better times. Average conditions prevailed when the remaining censuses were taken. From the census reports one can infer that so far as the urban population is concerned there was very little

With such a large migration constantly taking place, any attempt to calculate a standardized death rate is bound to result only in an approximation. Nevertheless the attempt is justified since death registration in Hongkong is accurate, and the census returns are very fairly accurate, while no such calculation has hitherto been made either for Hongkong or for any Chinese city.

The results I have obtained, though not as accurate as similar data for Western European countries or North America, are I believe reasonably near the truth.

This paper discusses only the urban Chinese of the colony. While personally scrutinizing each of the 420,000 death returns dealt with in this paper, I excluded those occurring among the non-urban population, such as the country dwellers, harbour inhabitants, those passing through the colony and also those of non-Chinese. Similarly census figures concerning only the urban Chinese of Victoria and Kowloon were used, and not those for the whole colony. It is necessary to emphasize this, because all official returns dealing with disease and deaths in the colony relate to the colony as a whole, and up to the present no attempt has been made to consider one section of the population alone; from a statistical point of view such official data are of little value.

Victoria throughout the last 50 years has been very overcrowded, and is no less so to-day than in the past. Its 740 acres of built-over land house over 400,000 people. In Kowloon the greater proportion of its 250,000 inhabitants live in an area accommodating over 300 to the acre. Victoria and Kowloon are in many respects one city separated by a mile of harbour; in this paper they will be taken together as such when dealing with data referring to 1901 or later. Prior to that date, Kowloon was too small to be considered an urban area, and I have not included it in my figures for 1897 to 1900. Whenever the name Hongkong is used in this paper, it refers to the urban areas I have mentioned, and not to the colony as a whole.

The Census Populations.

Table I shows the growth and the structural changes which have taken place in the urban Chinese population of Hongkong since 1897. In that year the first census sufficiently accurate for the purpose was taken. All figures are adjusted to estimated mid-year populations in the census years.

In the early years of the colony, *i.e.*, in the 'forties and 'fifties of last century, Hongkong from the Chinese point of view was a place to which the young adult male came to seek a living; but from which, after the best years of his life were over, he departed to rejoin his wife and children who had been left behind in the ancestral village up country. This might lie anywhere in the neighbouring provinces of China. By the middle of the 'eighties some of the labouring classes began to bring their brides or families to Hongkong to live, after the

husbands had found an occupation. By the turn of the century this tendency was well marked, and has continued at an ever increasing rate. As conditions became more favourable for the growth of family life, and incidentally as the standard of life improved, this tendency to bring relatives and dependents to Hongkong became the rule: no longer were children, born in Hongkong, sent back in large numbers to the ancestral village to live until old enough to be married or to earn a living.

TABLE I.

HONGKONG URBAN CHINESE POULATIONS AT SUCCEEDING CENSUSES, 1897-1931, WITH THE PERCENTAGE OF THE POPULATION IN EACH AGE GROUP.

Age in Years.	1897	1901	1911	1921	1931
0 upwards	4·42	3·94	4·70	7·78	8·59
5 ,,	5·37	4·81	6·15	7·03	7·44
10 ,,	6·27	6·16	6·84	8·85	8·14
15 ,,	25·53	27·55	24·30	25·33	25·21
25 ,,	25·71	26·92	26·06	22·43	21·21
35 ,,	17·05	16·58	17·82	15·81	14·57
45 ,,	10·33	9·07	8·93	8·05	8·84
55 ,,	4·13	3·84	3·77	3·28	4·33
65 ,,	0·98	0·90	1·15	1·23	1·31
75 ,,	0·19	0·20	0·27	0·20	0·40
85 ,,	0·01	0·04			
Totals—					
Males	117,544	163,169	195,322	272,153	371,906
Females	45,531	57,150	90,796	162,571	268,850
Persons	163,075	220,319	286,118	434,724	640,756
Intercensal increase		57,244	65,799	148,606	206,032

These changes show themselves in the figures of Table I, where it is seen that a steady increase has taken place in the relative and absolute numbers of the very young and the elderly during the last 40 years. There has always been a preponderance of males, though now much less than in the past.

There are two points to which attention should be drawn in these census figures. In those for 1911 there are stated to be included about 20,000 refugees who had fled from the revolution which resulted in the destruction of the Manchu dynasty. The census in 1921 was taken at a time of poor trade conditions when a certain unknown number of people would have returned to their homes up country to await better times. Average conditions prevailed when the remaining censuses were taken. From the census reports one can infer that so far as the urban population is concerned there was very little

evasion when filling in the returns except for infants, a deficiency which will be discussed later in the paper. Table I shows that there has been a gradual increase in the percentage of the age group 0–4 years. One reason for this has already been mentioned, namely, the gradual increase in family life, with the resulting increase in the number of children. Another reason, also mentioned, was the diminishing tendency to send children back to the ancestral village with the passage of time. The small percentage of children among Chinese families may come as a surprise to some. Contrary to general belief, Chinese families are not large.* In the age groups 5–9 and 10–14 years, the percentages have also risen throughout the period. Throughout working life, *i.e.*, in the age groups from 15 to 44 years of age, there has been some decline in the proportions.

After the age of 45 years the percentages are very low and much less than in Britain. One reason for this is the very much shorter expectation of life among Chinese. Old people, namely those over 60 years of age, are rare in China, even in country places. (This is my impression. I hope to prove it statistically for a rural community in a later paper.)

THE DEATH RATES AT ALL AGES.

Table II shows the standardized death rates for the urban Chinese population at each of the censuses since 1897 (directly standardized against the England and Wales population of 1901). The crude rate is also given for each year. With a very fluctuating population such as that of Hongkong it is not possible to work out accurate standardized rates for intercensal years, and for the calculation of the crude death rates I assumed that in intercensal years the population increased in geometrical progression.

During the first two-thirds of the period under review Hongkong was afflicted with epidemics of plague which caused great variations in the death rate from year to year. Since 1922 the disease has been absent.† Additional factors contributing to the fluctuations are the smallness of the colony's population, and the habit of the Chinese, mentioned elsewhere (UTTLEY, 1938) of returning to their native village when they consider themselves sick enough to die. This last factor will tend to reduce the rate to a value lower than it otherwise would have been, especially in an epidemic year, because

*LENNOX (1919) in dealing with a series of 4,000 fathers, states that in Peking there are 2·1 children per family, 1·4 being alive and 0·7 dead. GRAY, quoted by LENNOX, working on another group in Peking of 1,000 mothers, found the corresponding figure to be 2·3 living children per family. HAMMOND and HSU (1927) when dealing with a series of 903 families, also in Peking, obtained a figure of 3·7 per family. FAN (1933) in Tsinan, Shantung, with 2,500 families found an incidence of 3·4 living births per family.

†Except for one death in 1928 and two in 1929.

TABLE II.

STANDARDIZED AND CRUDE DEATH RATES. HONGKONG, 1897-1936.
(England and Wales standardized death rate for comparison.)

Year.	Hongkong.				England and Wales.	
	Standardized Death Rate.			Crude Rate.	Standardized Death Rate.	
	Male.	Females.	Persons.	Persons.	Males.	Females.
1897	33·9	39·0	37·3	20·7	18·8	15·9
1898				24·0		
1899				26·3		
1900				28·1		
1901	38·3	54·9	46·7	26·0	18·5	15·5
1902				23·9		
1903				21·4		
1904				20·9		
1905				21·8		
1906				26·0		
1907				23·1		
1908				30·2		
1909				22·6		
1910				22·9		
1911	42·3	49·7	46·0	24·4	15·6	13·0
1912				28·6		
1913				28·1		
1914				25·1		
1915				20·4		
1916				26·3		
1917				25·0		
1918				30·6		
1919				27·3		
1920				27·0		
1921	33·2	32·4	33·0	23·7	12·5	10·2
1922				27·7		
1923				27·5		
1924				28·8		
1925				27·8		
1926				22·4		
1927				25·4		
1928				24·9		
1929				27·4		
1930				28·8		
1931	40·0	35·9	36·7	30·7	11·4	9·2
1932				27·8		
1933				26·6		
1934				24·9		
1935				26·9		
1936				31·7		

Chinese are easily alarmed by an epidemic, and if they can afford to do so they try to get away from an infected area to their homes for the time being.

A consideration of Table II shows that the standardized rate of males was less than that of females up to and including 1911. In 1921, the rates were the same for the two sexes, in 1931 the male rate was 4·1 per 1,000 higher than the female rate. The rates both for persons and for the sexes separately have varied considerably from one census year to the next and show no definite trend. Concerning certain of the years it must be pointed out that 1901 was a year in which a severe outbreak of plague accounted for a fifth of all deaths. In 1906, there was a similar though less severe epidemic which caused a tenth of all deaths. In 1908 plague and smallpox between them accounted for an eighth of all deaths. 1911, 1912 and 1913 were plague years, 1912 and 1913 smallpox years, the deaths from these causes averaging about a tenth of the total each. In 1918, there was the influenza pandemic, in addition to which cerebrospinal meningitis visited the colony for the first time in epidemic form and caused 9 per cent. of all deaths ; a large fire accounted for another 5 per cent. and plague was also somewhat severe.

On the other hand, there was no epidemic of any disease in 1897, 1903–4–5, 1909, 1910 and 1915. With reference to the relatively high crude death rates for the last 15 or 20 years it must be remembered that in the earlier years the population contained a larger proportion of people aged 5 to 44 years than it did later, and consequently favoured a lower crude rate in the first half of this period than it did in the latter half.

THE DEATH RATES AT AGES.

Table III shows the death rates at ages for each of the five census years. Similar figures for England and Wales in 1931 are given for comparison.

It must be remembered that the 1901 figures are unduly high because of the severe epidemic of plague that occurred in that year. Taking first the rates on both sexes combined it will be seen that there has been relatively little change in the enormously high death rate during the first five years of life. It seems to have been at its highest in 1911, but too much reliance must not be placed on this rate of early childhood because a certain unknown number of infants are not registered at the censuses. This applies more especially to female infants (see later). In the second 5 years of life, there has also been little change ; the figures suggest a slight fall. At ages 10–14 the rate has declined by half, the fall taking place entirely in the latter half of the period. At 15–20 years there has been a well marked drop since the peak in 1901, but little improvement between 1897 and 1931. At all later ages there has been not only no improvement in the mortality experience but a rising rate in the last 20 years. (The figures for 75 years of age and upwards are unreliable owing to the smallness of the numbers involved.)

TABLE III.

DEATHS AT AGES PER 1,000 LIVING AT SUCCESSIVE CENSUS YEARS, HONGKONG, 1897 TO 1931.

(P. = persons; M. = males; F. = females.)

	1897			1901			1911			1921			1931			England and Wales, 1931		
Age.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.
0 —	190	164	211	209	159	258	283	257	310	165	165	164	199	198	201	20·0	22·4	17·5
5 ,,	15	14	15	25	18	29	17	15	18	14	13	15	13	13	14	2·1	2·3	2·0
10 ,,	8	8	9	17	13	22	8	7	9	5	3	6	4	4	4	1·5	1·5	1·5
15 ,,	6	5	10	12	10	21	8	4	9	5	4	6	5	5	6	2·5	2·6	2·4
20 ,,	8	8	10	12	11	15	7	6	10	6	6	6	9	9	8	3·0	3·2	2·9
25 ,,	10	10	11	16	15	18	9	8	10	9	9	9	11	12	10	3·4	3·5	3·3
35 ,,	14	15	11	18	18	15	12	12	10	13	14	11	15	18	10	5·1	5·8	4·5
45 ,,	19	22	12	22	22	22	17	19	12	20	22	15	24	29	16	9·8	11·6	8·2
55 ,,	30	33	22	42	43	40	28	30	25	39	46	28	45	56	31	20·2	33·5	17·3
65 ,,	62	66	58	08	103	114	55	57	53	70	74	63	117	127	107	52·9	60·5	46·6
75 ,,	179	142	203	217	146	273	145	160	175	225	267	204	80	126	65	128·3	141·4	119·5
85 +	304	200	333	239	290	213										285·5	291·2	282·6

Chinese are easily alarmed by an epidemic, and if they can afford to do so they try to get away from an infected area to their homes for the time being.

A consideration of Table II shows that the standardized rate of males was less than that of females up to and including 1911. In 1921, the rates were the same for the two sexes, in 1931 the male rate was 4·1 per 1,000 higher than the female rate. The rates both for persons and for the sexes separately have varied considerably from one census year to the next and show no definite trend. Concerning certain of the years it must be pointed out that 1901 was a year in which a severe outbreak of plague accounted for a fifth of all deaths. In 1906, there was a similar though less severe epidemic which caused a tenth of all deaths. In 1908 plague and smallpox between them accounted for an eighth of all deaths. 1911, 1912 and 1913 were plague years, 1912 and 1913 smallpox years, the deaths from these causes averaging about a tenth of the total each. In 1918, there was the influenza pandemic, in addition to which cerebrospinal meningitis visited the colony for the first time in epidemic form and caused 9 per cent. of all deaths ; a large fire accounted for another 5 per cent. and plague was also somewhat severe.

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0 —	190	164	211	209	159	258	283	257	310	165	165	164	199	198	201	20·0	22·4	17·5
5 „	15	14	15	25	18	29	17	15	18	14	13	15	13	13	14	2·1	2·3	2·0
10 „	8	8	9	17	13	22	8	7	9	5	3	6	4	4	4	1·5	1·5	1·5
15 „	6	5	10	12	10	21	8	4	9	5	4	6	5	5	6	2·5	2·6	2·4
20 „	8	8	10	12	11	15	7	6	10	6	6	6	9	9	8	3·0	3·2	2·9
25 „	10	10	11	16	15	18	9	8	10	9	9	9	11	12	10	3·4	3·5	3·3
35 „	14	15	11	18	18	15	12	12	10	13	14	11	15	18	10	5·1	5·8	4·5
45 „	19	22	12	22	22	22	17	19	12	20	22	15	24	29	16	9·8	11·6	8·2
55 „	30	33	22	42	43	40	28	30	25	39	46	28	45	56	31	20·2	33·5	17·3
65 „	62	66	58	08	103	114	55	57	53	70	74	63	117	127	107	52·9	60·5	46·6
75 „	179	142	203	217	146	273	145	160	175	225	267	204	80	126	65	128·3	141·4	119·5
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(P. = persons; M. = males; F. = females.)

	1897			1901			1911			1921			1931			England and Wales, 1931		
Age.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.
0 —	190	164	211	209	159	258	283	257	310	165	165	164	199	198	201	20·0	22·4	17·5
5 „	15	14	15	25	18	29	17	15	18	14	13	15	13	13	14	2·1	2·3	2·0
10 „	8	8	9	17	13	22	8	7	9	5	3	6	4	4	4	1·5	1·5	1·5
15 „	6	5	10	12	10	21	8	4	9	5	4	6	5	5	6	2·5	2·6	2·4
20 „	8	8	10	12	11	15	7	6	10	6	6	6	9	9	8	3·0	3·2	2·9
25 „	10	10	11	16	15	18	9	8	10	9	9	9	11	12	10	3·4	3·5	3·3
35 „	14	15	11	18	18	15	12	12	10	13	14	11	15	18	10	5·1	5·8	4·5
45 „	19	22	12	22	22	22	17	19	12	20	22	15	24	29	16	9·8	11·6	8·2
55 „	30	33	22	42	43	40	28	30	25	39	46	28	45	56	31	20·2	33·5	17·3
65 „	62	66	58	08	103	114	55	57	53	70	74	63	117	127	107	52·9	60·5	46·6
75 „	179	142	203	217	146	273	145	160	175	225	267	204	80	126	65	128·3	141·4	119·5
85 +	304	200	333	239	290	213										285·5	291·2	282·6

The general trend of the rates of males and females has been fairly similar. In the years of childhood, 0–5 and 5–10, neither shows any definite improvement over the 34 years. At 10–15 both sexes show a declining rate and with females this improvement also extends to the age groups 15–20 and 20–25. At ages 25–45 both sexes show a rate that is nearly stationary over the period while at ages over 45 both show a rising mortality, the increase being, however, substantially greater amongst the males.

The ratios of the male to the female rates at each age and at each period are shown in Table IV. The figures for ages 0–4 show at first higher values

TABLE IV.

RATIO OF MALE TO FEMALE MORTALITY.

$$i.e.\ \frac{Male\ death\ rate \times 100^{*}}{Female\ death\ rate\ at\ Ages}$$

Age Group.	1897	1901	1911	1921	1931	England and Wales, 1931.
0	77	62	83	101	99	128
5	93	62	82	87	93	115
10	89	59	77	50	100	100
15	47	48	46	67	83	108
20	79	73	57	100	113	110
25	95	83	80	100	120	106
35	136	120	126	127	180	129
45	188	100	155	147	181	141
55	146	108	119	164	181	136
65	114	90	106	117	119	130
75	67	64	57	131	194	112

*These figures do not always correspond exactly with those derivable from Table III owing to the omission of decimals in that table.

for females than for males ; in the last two census years the rates were equivalent. It has already been pointed out that the figures for the first few years of life must be accepted with some reserve. I believe, however, that the infant mortality of females in China has always exceeded that of male infants, if only because of the much greater care bestowed on the latter in Chinese homes.

Female infants are usually valued less than males (LENNOX, 1919 ; HSU and CHU, 1930).

At the ages 5–19 the rate of males is less than that of females ; and 20–34 in the earlier years this was also found, but now the reverse holds. At ages 35 and onwards the rate of males has always exceeded that of females, except possibly in old age. A reasonable explanation for the lower death rate of young adult Chinese females in Hongkong is to be found in the fact that so many young and healthy brides and wives are constantly coming into the colony from the country. To a certain, though limited, extent their life in the home is less strenuous and less exposed to the unhealthy influences outside the home which are the lot of their husbands in the shop, workshop and in the carrying trades that absorb so large a number of the coolie class.

COMPARISON OF THE HONGKONG RATES IN 1931 WITH THOSE FOR ENGLAND AND WALES.

Table III shows that the greatest difference between these eastern and western experiences lies in the first 10 years of life, the ages at which the eastern lack of knowledge of antenatal and postnatal care and of the elementary rules of hygiene tells so severely on mortality rates. The very severe degree of over-crowding in the tenements, the closing of all windows and doors in the cold weather and often in the hot weather as well, the promiscuous use of chopsticks and rice-bowls, the universal habit of spitting on floor and street, the habit many mothers have of chewing their children's food before inserting it into their mouths, the very poor quality food of the coolie class, with its associated avitaminosis, the intense struggle for existence which compels a family to make each child work as soon as it can walk, so as to increase the family's wage, all tell on the health of the growing infant and child and result in the death of many who in England would recover and be healthy citizens. It is not surprising that the rate is so high, rather is one surprised that it is not much higher.

Throughout the years of working life, from puberty to 54 or thereabouts, the rates are only from two to three times the British rates. I believe that these relatively low rates are produced by the constant train of new and healthy arrivals, already referred to, who come seeking work. The low rates are, therefore, more apparent than real, because if it were possible to compile rates dealing only with those who have been born and bred in the city of Hongkong, they would certainly be higher.

At the ages of 55–74 the rates which in 1897 were not much above the British figures for 1931, had by the latter date risen to approximately twice the value. This rise is not seen in the younger years of adult life when people as a whole are healthier, but the increasing overcrowding of the city has resulted

in an increase of the infirmities and sicknesses of old age and, therefore, an increase in mortality at these ages.

The Mortality at ages 0–5.

During the last 2 years registration of births in Hongkong has been much more strictly enforced and now probably all births are registered. In the past there was a certain amount of evasion of birth registration, whereas there was no evasion of death registration. This results in an apparently very high death rate for the first quinquennium of life, and makes it impossible to calculate an infant mortality rate with any reasonable degree of accuracy. I do not consider, however, that this evasion has ever exceeded 20 per cent. of all births in urban areas, and I think that 10 per cent. would be a more likely figure. Even if we accept the higher figure and assume that the death rate in the first 5 years of life in 1931 was in the neighbourhood of 170 per 1,000 living, instead of the recorded rate of 200, we still have a rate eight or nine times that of England and Wales ; 49·8 per cent. of the deaths of males and 63·6 per cent. of the deaths of females in Hongkong in 1931 belonged to these first 5 years of life.

The Seasonal Incidence of Deaths.

Table V shows the monthly distribution of deaths in different intercensal periods. I have assumed a standard month of 31 days ; the average number of

Table V.

Percentage monthly distributions of Deaths in Hongkong, 1897-1936.

Month.	1897–1900	1901–1910	1911–1920	1921–1930	1931–1936	1897–1936
January	79·5	75·6	88·1	87·2	86·3	84·6
February	92·5	77·4	89·3	91·3	88·4	87·6
March	92·4	81·8	88·6	88·8	93·1	88·3
April	110·7	98·3	96·8	97·8	101·1	98·3
May	140·1	126·1	114·6	104·6	110·3	112·0
June	127·3	136·4	128·4	108·1	104·6	116·4
July	108·7	111·4	113·5	114·2	107·8	110·9
August	92·3	110·3	98·2	108·3	107·8	104·5
September	91·0	98·4	100·3	101·9	101·7	111·3
October	92·6	101·7	98·0	99·9	103·1	99·5
November	96·6	93·2	95·1	99·2	96·8	96·0
December	84·6	88·0	88·6	95·9	99·3	92·9
Total number of deaths	16,837	59,455	93,360	137,576	113,037	420,265

deaths in this period (*i.e.*, the yearly total divided by 12) I have called 100 and have expressed the deaths registered in each month in proportion to this. The table shows that there is a uniform tendency for more deaths to occur in the months May to September, than at other times. January is the healthiest month, in that fewest deaths occur then. The autumn and winter months are cool, there is little rain and the climate is very healthy at that time. Against this must be put the fact that in the cool weather people are huddled together at night in their tenements and all windows are closed. In the summer large numbers sleep out of doors, with a consequent lessening of the overcrowding. With the advent of summer a minimum of clothes is worn, all winter ones are put away and plenty of light and air can get to the skin. With the arrival of the first cold spell winter clothes are unpacked or taken from the pawnbroker, and any fomites present are liable to cause outbreaks of smallpox or other infectious fevers. The presence of intestinal diseases during the summer months and such conditions as malaria, which have their greatest incidence in the summer, will account for the seasonal increase seen in the table. The fall in recent years from the high figures previously recorded for May and June is due to the disappearance of plague.

SUMMARY.

(1) The standardized death rate of males living under urban conditions in Hongkong has varied between 1897 and 1931 from 33·2 to 42·3 per 1,000 living, of females from 32·4 to 54·9, and of persons from 33·0 and 46·7. Without a radical change in the conditions prevailing in the city as described in this paper, one cannot expect any great lowering of this high rate.

(2) Owing to deficiencies in registration the infantile and early childhood death rates cannot be calculated with accuracy. Mortality at ages 0–4 appears to be in the neighbourhood of 170 per 1,000 living or at least eight times the rate in England and Wales.

(3) There appears to be a higher death rate for females than for males up to the age of 20, while beyond this age, and especially after age 35, the rates of males are the greater.

(4) In every age group the death rate in Hongkong in 1931 is considerably greater than the rate in England and Wales in the same year, being never less than two to three times as high.

(5) In 1931, 49·8 per cent. of all male deaths and 63·6 per cent. of all female deaths occurred in the first quinquennium of life.

(6) A larger proportion of deaths occurs in the five months, May to September, than at other times of the year.

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CORRESPONDENCE.

BEJEL.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

Dr. RICHARDS' interesting letter on bejel, which you published in the November issue, and a carbon copy of which Dr. RICHARDS kindly addressed to me, raises several important points. Perhaps I may be permitted to reply to one or two where confusion may have arisen.

As to the word 'bejel,' my advocacy of its use grew out of a realisation that this type of syphilis would never be clearly seen as a clinical entity until it had been properly *labelled.*

It seemed to me that a clear distinction had to be made between venereally acquired syphilis and the acquired syphilis of childhood. As long as the word 'syphilis' was used for *both,* even with qualifying adjectives, there would be confusion in medical thought. What I wanted was a term which would gather up in one word the whole intricate picture of non-venereal syphilis, all the qualifying and descriptive adjectives which had been applied to it—a single word which would evoke that specific concept in the mind of reader or listener. It was appropriate and inevitable that I should select as this label the word bejel which the Arab villagers themselves use to differentiate their childhood syphilis from the " syphilis of the foreigner " (franghi). None of my writings give ground for inference that bejel is being introduced as the name of a separate disease ; but in justifying my inclusion of the word bejel in medical nomenclature, I might have made plainer that I was not using nomenclature in the technical taxonomic sense. I believe that bejel is caused by infection with *T. pallidum,* and is therefore a type of syphilis.

Now if the word bejel is a *label,* it has no longer any geographical moorings and can be legitimately used in the sense of a label anywhere this type of syphilis is found ; and it can be used in medical thought and literature as a useful term to describe a certain clinical entity. I cannot, therefore, share any apprehension that it can be confused with the " bagl " of the Sudanese which, if it should appear in medical literature, would properly be referred to as gonorrhoea.

Dr. RICHARDS has laid us in his debt for the information he gives about the word bejel. The meaning of " ulcers " would seem more suitable to syphilitic than to gonorrhoeal conditions.

As I have not met gonorrhoea among the semi-nomad Bedouin Arabs, I have not had occasion to learn whether they have a colloquial word of their own for gonorrhoea. I suspect not, and rather imagine they would give it the generic name for a " discharge," *nizl* or *nazool,* or the word used for gonorrhoea in the town of Deir-ez-Zor and in the other parts of Syria and the Lebanon, namely, *ta'aqibah,*

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he says, it was the best that could be done in the circumstances—but it was done 25 years ago.

For the settlement of the question of microfilarial periodicity in this infection Dr. MANSON-BAHR feels that two things are needed—many more detailed observations, and the discovery of a suitable experimental animal. As to the second, the well-known reluctance of parasitic helminths to lead fully normal lives in more than one host species, or at most in a few of them, may well mean that insistence on this second need will put off the settlement of this important practical point for long, perhaps for ever; this cannot be Dr. MANSON-BAHR'S wish. It becomes necessary, then, to concentrate on the need for many more detailed examinations, and it is reassuring to feel that here we are in complete agreement. What follows is an account of my own share in trying to get this done. Professor O'CONNOR has left me by Will his microscope slides, and if all those in his laboratory in New York at the time of his death were his to give, the number at my disposal will be great; and his letters to me during the past 7 years show that they concern many persons. I propose to set out a typical demonstration of these slides at this Society's Laboratory Meeting next March, so that they may come under the observation of many. Further, I propose, so far as the material at my disposal goes, to distribute similar sets of these (perhaps a dozen sets in all, apart from those which will be needed for the United States) to workers and teaching centres concerned with tropical medicine in different countries. My reasonable mental reservation in parting with this valuable material (so making it available for more leisurely examination by others) is that it shall go where it will in my view be used with open mind to further the spread of knowledge for its own sake, and that it shall in this way furnish a deserved tribute to the memory of the fine and indefatigable worker by whom they have been entrusted to me. Again, I have interested the heads of two Schools of Tropical Medicine within the Empire and have thus, I am satisfied, insured that O'CONNOR'S great work shall be repeated. All this I had done before, in some cases months before, Dr. MANSON-BAHR'S letter was written, in order to make it as certain as I may that many more detailed observations shall be made on this matter. With the great opportunities open to him through his position at the London School of Hygiene and Tropical Medicine, no doubt Dr. MANSON-BAHR has, necessarily on rather different lines, done as much as I have to insure that there shall be made that adequate and essential examination of what he regards as the cornerstone of tropical medicine. I again deprecate the mixing of helminthology and poetic imagery, but if his cornerstone simile is a good one, and the whole structure of tropical medicine accordingly imperilled by a doubt about the soundness of views on the cause of microfilarial periodicity, Dr. MANSON-BAHR will, of course, increase to the utmost such efforts as he may now be making to put this matter to the test.

I am, etc.,

CLAYTON LANE.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOL. XXXI. No. 5. MARCH, 1938.

Proceedings of an **Ordinary Meeting** of the Society, held at Manson House, 26, Portland Place, London, W.1, at 8.15 p.m., on

Thursday, 20th January, 1938.

Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.), *President*, in the Chair.

PAPERS.

(1) THE TREATMENT OF ACUTE FULMINATING CARDIAC BERIBERI (SHÖSHIN) - - - - By R. BRUNEL HAWES

(2) THE PHYSIOLOGY AND BIOCHEMISTRY OF VITAMIN B_1 By R. A. PETERS

(3) BIOCHEMICAL CHANGES, ESPECIALLY OF PYRUVIC ACID, IN RELATION TO SOME CLINICAL FEATURES OF BERIBERI - - - - - - - - - By B. S. PLATT

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THE TREATMENT OF ACUTE FULMINATING CARDIAC BERIBERI (SHÖSHIN).

BY

R. BRUNEL HAWES, M.B., F.R.C.P.

Professor of Medicine, King Edward VII College of Medicine, Singapore.

The knowledge of the vitamins and other food factors has advanced so rapidly of recent years, and has opened up such enormous possibilities of treating and preventing diseased states, that it becomes of the utmost importance to describe and define as accurately as possible the gross manifestations of pure deficiencies and the manner of their reaction to specific treatment before profitable progress can be made with minor maladies.

I must therefore first describe as closely as possible what I wish to speak about tonight.

I intend to refer only to the acute fulminating dying beriberi or shöshin. The type of patient that comes for treatment desperately breathless, restless, throwing himself about in bed, groaning with pain in the epigastrium, tries to sit up, tries to lie down flat, on his right side, then on his left side, finding no ease in any position, vomits, demands water, vomits again. Often pulseless with engorged veins, particularly in the neck, and within a few minutes to 6 hours falls back and dies, intensely dyspnoeic to the end.

The heart is greatly enlarged, particularly to the right; this enlargement of the right auricle and ventricle comes on very quickly and is easily demonstrable by percussion.

The pulse rate rises to perhaps only 120 to 130, never so rapid as in many acute toxic conditions, the systolic pressure often remains normal and only drops in the very late stages; the diastolic pressure, however, always drops with the onset of severe symptoms, and in dying patients pressure frequently cannot be determined as pistol-shot sounds are audible in the uncompressed arteries. These sounds and the presence of capillary pulsation give some resemblance to aortic regurgitation although no other resemblance to this condition is present.

Just before death the systolic pressure drops also, and the veins (except the veins in the neck) collapse, so that in some of our cases no record of blood pressure could be made of the collapsed pulseless patient.

I am speaking then, *not* about the mild case or severe case or the nervous case, but about the dying case.

In 1928 Professor JANSEN sent me ten phials of the crystals of vitamin B_1 that he had isolated and made up for injection. I tried these on dying patients with dramatic results, the same patients in a few hours showing a complete

clinical recovery: in others there was a temporary amelioration of symptoms although they subsequently died.

I wrote congratulating Professor JANSEN, giving him my results and asking for further supplies. He replied, saying that Dr. AALSMEER, of Soerabaia, had observed temporary flushing and dizziness with the injections, and therefore he had purified his extract further and sent me a supply of the purified product. With these I had no results at all; they did not seem to have the slightest effect.

On writing again suggesting that something in the purification had destroyed the activity, Dr. DONATH promised to prepare ampoules of pure crystalline vitamin in weighed amounts. Unfortunately, Dr. DONATH became ill and had to return to Holland and our collaboration ceased as the further products had no apparent effect in dying patients.

It was suspected, as is now well known, that the reaction was quantitative. Professor ROSEDALE prepared for me enormous quantities of a concentrated extract, which I gave by the mouth or rather forced into the stomach of these severe cases—again with no result. Not realizing that to obtain any result large doses must be given by injection, we endeavoured to discover what was missing in the extract; for, having seen the initial results, I refused to believe what has so often been postulated, namely, that incurable damage had occurred in these cases.

MOURIQUAND divided the disease into three stages: Imperceptible, incomplete fastigium and incurable fastigium, and it was the last that I hoped to prove did not exist in the human, any more than in the laboratory animal. Eventually the advent of the synthetic products of WILLIAMS so lessened the cost that I was able to afford large doses in all our cases. Clinically, after the injection of an adequate amount, in a few minutes the moribund patient becomes less restless, and often drowsy, as if the great discomfort was now disappearing and exhaustion taking its place. Yet not for an hour or more is any effect noticed in the pulse rate or on the systolic or diastolic pressure. In fact, in a few cases, the patient states that he now feels quite comfortable and is no longer breathless, yet the pulse rate and blood pressure remain unaltered and do not show any change for many hours.

If insufficient vitamin has been given, shortly after a temporary amelioration of symptoms, there is a return of dyspnoea or a sudden collapse and death. On the other hand, however, if sufficient has been given, after the patient has recovered from his exhaustion, he states that he feels quite well again and in a few days is able to leave hospital.

In many cases the recovery is extremely dramatic and I well remember my shock when first using JANSEN and DONATH'S crystals, on seeing, when returning to a ward in the afternoon that a dying man whom I had injected with them in the morning had waddled away out of his bed the whole length of the ward to get a drink of tea.

The dosage necessary seems to vary from case to case: Table I illustrates the enormous amount sometimes needed.

TABLE I.

RESPONSE OF VASOMOTOR SYSTEM TO B_1.

Case No.	Duration of Dyspnoea.	Dosage.	Blood Pressure and Pulse Rate.							Blood Urea.		Diuresis in 7 days.	
			Before.	1 hour after.	2 hours after.	4 hours after.	24 hours after.	48 hours after.	7 days after.	Before.	2 days after.		
B.										Mg. %	Mg. %		
B.4	4 days	1,600 p.u.	124/28 160	122/28 158	118/30 152	118/32 146	114/40 120			56	50	Intake Output	
B.6	5 „	1,600 p.u. 800 p.u. 12 hours later 800 p.u. 24 hours later	95/45 88	86/40 82	84/40 80	90/50 92	108/80	120/90 74	135/100	146	132	Intake Output	339 ozs. 553¾ „
R.													
R.17	4 „	1,500 i.u. 1,000 i.u. 1,000 i.u.	70/35 130	70/35 116	70/45 136	70/46 146	118/82 106	120/94 78	110/90 84	59	77	Intake Output	224 „ 255 „
R.24		2,000 i.u. 1,000 i.u. 11 hours after					90/75	116/100	112/85			Intake Output	220 „ 263 „
S.B.													In 4 days
S.B.1	3 „	1,600 p.u. 800 p.u. 1,500 i.u. (Java)	65/40 122	60/40 114	80/40 116	80/40 110	70/57	116/90		77	95	Intake Output	171 ozs. 14 grammes

Notes on Tables.—Though as yet there is no final agreement on exact relationship, approximately:
1 mg. B_1 crystal = 250 — 300 international units (i.u.) = 150 pigeon units (p.u.)
B = Betaxin; R = Roche; S.B. = Synthetic Betaxin; J = Java; G = Geneeskundig Laboratorium.

Betaxin.

B.4. This patient died within 48 hours of injection. He seemed to improve, became less dyspnoeic and cyanosed, pulse rate down from 150 to 120 but suddenly collapsed and died. A type of death from insufficient dosage.

B.6. Very severe case, dyspnoeic and cyanosed, vomiting, restless, pulse feeble but heart fairly slow with extrasystoles, pretibial oedema; 6 hours after very restless, no improvement in heart and blood pressure; 12 hours after quiet but still no improvement in blood pressure and pulse, still vomiting; 24 hours, considerable improvement marked after third injection.

Roche.

R.17. No improvement after 1,500 units. Another injection 1,000 units 6 hours after. Condition: blood pressure and pulse improved. Remarkable recovery due to second injection; 8 days later another 1,000 units given to accelerate progress; 5 days later able to walk with support. Discharged after 21 days in hospital. B.P. maintained.

R.24. Very severe case with typical signs; restlessness marked, B.P. could not be recorded, unable to obtain blood from veins for examination. The injection had to be given into the jugular vein; 8 hours later considerable improvement, pulse could now be felt.

Synthetic Betaxin.

S.B.1. Severe case, rapid weak pulse, dyspnoeic; 9 hours after still very distressed, pulse rate slower but blood pressure same, another 800 p.u. given; 21 hours after not so distressed, but blood pressure and pulse rate same; 24 hours later condition worse, body cold, pulse very feeble; Java 1,500 i.u. given; 2 hours after improvement noticed, but all the time urine output was very scanty, this did not improve with diuretics and cupping; 4 days after admission drowsy, some increase in blood urea—18 mg. × 100 c.c., blood pressure still high. Patient died. Postmortem: only feature very congested kidneys.

TABLE II.

RESPONSE OF VASOMOTOR SYSTEM TO B_1.

Case No.	Duration of Dyspnoea.	Dosage.	Blood Pressure and Pulse Rate.							Blood Urea.	
			Before.	1 hour after.	2 hours after.	4 hours after.	24 hours after.	48 hours after.	7 days after.	Before.	2 days after.
J.										Mg. %	Mg. %
J.2	3 days	1,500 i.u.	130/– 116	130/– 108	122/40 104	126/46 98	122/106 80	130/106 64	130/90 72		
J.3	2 "	1,500 i.u.	118/– 132	80/– 126	80/– 112	90/60 112	130/110 80	130/95 78	180/114 74		25
R.											
R.6	6 "	1,500 i.u.	110/– 126	110/– 124	110/– 122	115/30 110	115/60 100	110/60 98	112/50 80	82	101
R.7	1 day	1,000 i.u.	105/– 126	96/– 124	102/– 120	106/60 120	120/80 100	122/74 84	110/70 72	156	37
R.14	6 hours	1,000 i.u.	110/– 120	105/– 112	105/35 98	110/40 100	100/60 80	104/58 74	108/62 72	35	22
R.19	9 days	1,500 i.u.	130/– 90	136/48 80	140/62 78	154/86 71	180/110	180/120		30	20
R.20	3 "	1,500 i.u.	120/– 124	116/– 122	110/30 118	108/60 112	130/100 78	135/100 76		38	24
R.23	1 day	1,500 i.u.	85/– 88	110/46 100	110/46 104		110/70 74	156/110	135/95	154	
S.B.	3 days	1,500 i.u.	90/20 118	90/20 116	100/50 122	94/60 118	122/102 82	122/102 60		102	131
S.B.5		1,600 p.u.									
R.											
R.24		3,000 i.u.	No pressure	No pressure	No pressure	No pressure	90/75	116/100	112/85		

Java.

J.2. Considerable improvement within 3 hours; drowsiness developed 1 hour after injection; 7 days after able to walk about, gait steady, able to rise from squatting position, sluggish knee jerks. Complication: malaria.

J.3. Typical case. Great clinical improvement in condition within an hour—quiet, less cyanosed, not collapsed, pain in abdomen gone. Although as sometimes occurs the systolic pressure had fallen and the diastolic not yet reappeared, it was not measurable

until after 3 hours; 1 day after injection arterial wall hard and rigid as in hypertension—remained so for 5 days. On discharge 8 days later, reflexes still absent, steady gait, able to rise from squat.

Roche.

R.6. Considerable improvement in 4 hours; B.P. maintained for 2 weeks. Patient absconded after 16 days when he was able to walk fairly well. No reflexes. Complication: malaria.

R.7. Suddenly became dyspnoeic; improved in 4 hours after injection; blood pressure still maintained.

R.14. *Early* case improved rapidly. Complication: malaria.

R.19. *Not* a severe case, oedematous but not very dyspnoeic. Improvement in blood pressure 1 hour after treatment. Patient comfortable, reflexes absent but walked steadily.

R.20. Severe case, improved as usual. Blood pressure maintained.

R.23 Old case of cardiac beriberi and malaria (B.T.), treated with one dose of betaxin 7 months ago with good results. Very severe this time, pulse imperceptible, dyspnoeic. Partial improvement with Roche first injection, 4½ hours later restless again; steady improvement after second injection; but urine scanty and general anasarca, vomiting. Blood urea 154 mg. × 100 c.c. rose to 227 mg. in 11 days, then fell to 26 mg. in 1 month. Urine: albumin +, casts +, diuresis after calcium chloride on the 11th day. Oedema subsided, blood pressure maintained 1 month after. On discharge, urine: albumin casts nil; blood urea normal.

Synthetic Betaxin.

S.B.5. Severe case, improvement in general condition in 3 hours, but blood pressure and pulse rate did not improve till next day.

Hyperpiesia.

A most interesting reaction can be seen here; it is one that occurs in many patients but not all.

After injection of the vitamin the diastolic pressure continues to rise until within 24 hours it is often well over 100 mm. of Hg (R.19, 120) with a corresponding rise in the systolic. At the same time the arteries can be felt gradually from hour to hour becoming more rigid and harder until they feel exactly like the peripheral arteries of essential hypertension.

The heart shows no reaction and does not appear to labour, there is no heaving apex beat nor increased first sound. The rise appears to be solely due to hypertonus of the musculature of the arteries.

This lasts, maybe a week or more, when the diastolic pressure falls to normal limits.

TABLE III.

INFLUENCE OF B_T ON BLOOD PRESSURE.

	Before.	Dosage.	1 hour.	3 hours.	24 hours.	48 hours.	72 hours.	
Normal case	118/78	1,500 i.u.	118/76	118/74	114/75	120/78	118/78	No ill effect.
Aortic incompetence	135/40	1,600 i.u.	130/40	132/40	135/40	138/40	138/44	No effect.
Thyrotoxic goitre	128/64	1,500 i.u.	130/64	134/64	120/68		114/72	No effect.
Hypertension with cardiac beriberi	200/112	1,500 i.u.		205/115	210/122	210/118		Great improvement. No influence on B.P.
Chronic interstitial nephritis with cardiac beriberi	200/130	1,600 i.u.			186/128	210/120	212/120	Great improvement. No influence on B.P.

It was only natural that I investigated this curious action in other conditions; firstly to see if there existed any danger in the use of these large doses and secondly if the action could be used in treating other conditions.

A few of our results are given in Table III.

1. *Normal case.* No effect.
2. *Aortic regurgitation.* No hardening of the arteries, decrease of capillary pulsation or alteration of the pulse pressure.
3. *Thyrotoxic goitre.* No effect for good or ill.

All conditions and all diseases can be complicated by cardiac beriberi, and hyperpiesic states are no exception.

In these two cases, which were very dyspnoeic but did not really come into the shöshin group, in other words they probably had 2 days or more of life left, you will notice the diastolic pressure had not yet dropped.

The beneficial results of the injection were immediate and obvious, and you will notice that no effect on the blood pressure is observable, either in the essential or rather dietetic hypertension, or in the case of chronic interstitial nephritis.

The explanation therefore of this phenomenon remains obscure and suggests a secondary factor.

TABLE IV.

DIURESIS FOLLOWING B_1.

Case No.	Duration of Dyspnoea.	Dosage.		Fluid. 1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
J. J.10	4-5 days	1,500 i.u.	Intake	oz. 13	oz. 70	oz. 49	oz. 49	oz. 65	oz. 51	oz. 35
			Output	7	127	69	64	74	104½	82
S.B. S.B.1	3 „	1,600 p.u. 800 p.u. 1,500 i.u. (Java)	Intake	38	43	74	54			
			Output	1	1	9½	3½			
S.B.5	3 „	1,600 p.u.	Intake	23	53	28	29	30	23	25
			Output	8½	65	78	47½	72	37	26
R. R.20	3 „	1,500 i.u.	Intake	60	47	53	44	33	47	41
			Output	17¼	29¾	108¾	157	71¼	52½	63½
R.23	1 „	1,500 i.u. 1,000 i.u.	Intake	59	62	38	35	27	38	34
			Output	3	5½	10	6½	7	13	

There is a general misconception with regard to beriberi, which is still perpetuated in the name wet beriberi as opposed to dry beriberi.

Gross oedema, *i.e.* subcutaneous oedema, serous effusions or oedema in the lungs, are due to the functional failure of renal secretion consequent on the general vascular derangement and therefore their presence, duration or extent are a measure of so many factors such as the water intake, concomitant disease, loss of fluid from bowels, skin or lungs, etc., that they can bear no direct relationship to the clinical picture of shöshin and are no guide to the severity of the attack.

The swelling that is typical is a colloid interstitial swelling and does *not* cause a pitting oedema, this subcutaneous oedema is secondary and is not present in all cases.

The interstitial swelling occurs in muscle and gland tissues throughout the body, probably in nervous tissue as well, but the great increase in the pressure in the cerebro-spinal fluid may be due to the enormous venous congestion.

This interstitial swelling gives the appearance of enlarged muscles all over the body, particularly noticeable in the leg muscles and the rounded facial appearance due to the swelling of the parotids, while other glands are similarly affected.

In some patients, as shown in Table I, practically no diuresis occurs, in others, in which the kidney has been thrown out of action and subcutaneous oedema is present, diuresis occurs on the second day or later, in other words, well after the patient has made a clinical recovery from his cardiac attacks.

J.10. Diuresis occurred on the second day.
S.B.1. Died.
S.B.5. Diuresis on the second and third days.
R.20. Diuresis on the third day, long after he had apparently recovered.
R.23. Not until the eleventh day.

In oedematous patients where a diuresis cannot be established, death occurs with all the signs of a pseudo-uraemia.

TABLE V.

CHANGES IN BLOOD UREA AFTER B_1.

Case No.	Duration of Dyspnoea.	Dosage.	Blood urea.		Fluid.							
			Before.	2 days after.		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
S.B.			Mg. %	Mg. %		Oz.	Oz.	Oz.	Oz.	Oz.	Oz.	Oz.
S.B.2		1,600 p.u.	87	35	Intake	16	31	32	28	28	26	23
					Output	8½	18½	30½	60	51	48	40½
B.												
B.5		800 p.u.	71	35								
B.9		800 p.u.	80	42	Intake	37	24	30	23	34	52	38
					Output	19	25	19½	31½	22	52½	65
J.												
J.10	4-5 days	1,500 i.u.	75	45	Intake	13	70	49	49	65	51	35
					Output	7	127	69	64	74	104½	82
J.11		1,500 i.u.	61	115	Intake	8	23	28	30	48	48	28
					Output	19	22	12½	12	13		
R.												
R.9	4 „	1,000 i.u.	84	22	Intake	31	23	34				
					Output	39	85	124				
R.7	1 „	1,000 i.u.	156	37	Intake	52	50	64	54	40		
					Output	33	45	50	26	27		
R.16	7 „	1,000 i.u.	133	42	Intake	64	55	29	38	41	38	42
					Output	37½	46	48	49	32	56	44

Synthetic Betaxin. *Blood Urea.*

S.B.2. Fairly severe case, usual improvement.

Betaxin.

B.5. Not a severe case—no improvement in foot and wrist drop up to time of discharge 6 weeks later.

B.9. Average case, but response delayed till next day when B.P. rose up and pulse rate came down.

Java.

J.10. Severe case, general improvement in 4 hours, but blood pressure and pulse improved in 12 hours.

J.11. Severe case, developed dyspnoea whilst a patient in ward. Low pulse pressure, improvement noticed in 6 hours, developed diarrhoea with blood and mucus next day (bacillary dysentery)—probable cause of rise in blood urea, which was still high 4 days later, dropped to normal in 2 weeks.

Roche.

R.9. Usual improvement in 4 hours ; 1 month later blood pressure 118/72.

R.16. Same improvement noticed as usual, developed multiple liver abscesses and died. Postmortem : no signs of cardiac beriberi.

The kidney frequently shows a functional insufficiency, giving rise to scanty urine with albumin and casts and high blood urea.

Provided no complicating disease maintains the increased blood urea, this always falls as the volume of urine increases.

J.11 is a case in point as he was a severe case suffering also from bacillary dysentery and the blood urea did not fall to normal for 2 weeks, although the kidneys had started to function on the second day.

R.16. This case was suffering from multiple liver abscesses and eventually died 2 weeks after admission. Postmortem showed no signs of cardiac beriberi.

If the renal function is not re-established the patient soon dies.

TABLE VI.

EFFECT OF B_1 ON BLOOD SEDIMENTATION.

Case No.	Duration of Dyspnoea.	Dosage.	Day.	Total Red.	H. B. %	Colour Index.	Bld. Calcium. Mg. %	Bld. Sedimentation in 1 hour.
G.23	7 days	1,500 i.u.	1st	5,610,000	100	0·89	11·6	3 mm.
			2nd	4,910,000	100	1·02	11·2	3 mm.
G.27	1 month	1,500 i.u.	3rd	5,010,000	101	1·01	11·4	3 mm.
			1st	4,700,000	80	0·85	10·4	5·7 mm.
G.29	1 day	1,500 i.u.	2nd	4,750,000	80	0·85	9·9	5 mm.
			3rd	4,010,000	75	0·93		5 mm.
G.30	3 days	1,500 i.u.	1st	4,470,000	80	0·95	11·6	4 mm.
			2nd	4,210,000	85	1·01	11·6	5 mm.
			3rd					
G.35	1 month	1,500 i.u.	1st	3,900,000	75	0·97	10·2	
			2nd	3,740,000	75	1·01	10·2	3 mm.
			3rd	3,730,000	75	1·01	9·8	4 mm.
			1st	4,240,000	83	0·98	10	3 mm.
			2nd	4,580,000	90	1·00	10·6	6 mm.
			3rd	4,590,000	91	1·01	10·9	7 mm.

I put these figures up as it is frequently stated that a B_1 deficiency leads to anaemia and it was noticed that these patients had on the average as high red cell counts as the average among other patients in the wards. You will see that the red cell count does not fall much on the onset of diuresis. The sedimentation rate was generally lower than normal.

The explanation was suggested that it was due solely to abstraction of fluid from the blood stream ; these figures, however, do not confirm this, but more accurate measurements of blood volume are required. However, they are enough to show the gross error of that common diagnosis of anaemia due to deficiency of B complex. If the expression is used at all it might be (B complex $-$ B_1), which would be slightly less vague.

Conclusion.

The main facts that emerge from a study of a pure vitamin B_1 deficiency are :—

1. The reaction is quantitative, the vitamin is non-toxic.
2. The effect of one dose is lasting.
3. It must be given by injection to have any rapid effect.
4. The diagnosis of a pure B_1 deficiency can be confirmed by an injection of an adequate amount of the vitamin and the response to treatment is very rapid. In England this may be important in children in whom I suppose the pure deficiency is most likely to occur.

My thanks are due to the successive directors of the Geneeskundig Laboratorium, Batavia-Centrum, Drs. Jansen, Donath, Brug, van Veen and Mertens who have so willingly prepared carefully standardized extracts for my use, to Bayer's who have given supplies of their synthetic products and to my assistants Dr. C. E. Smith and Dr. E. S. Monteiro who at all hours have been ready to treat these urgent cases and make careful observations.

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BY

R. A. PETERS, *M.C.*, M.D., F.R.S.,

Professor of Bio-chemistry, University of Oxford.

A brief synopsis of some of the main features of vitamin B_1 deficiency in animals seems needed by way of introduction. The first symptom to be noticed is lack of appetite, followed naturally by loss of weight unless the animals are fed artificially. When the weight has fallen to some 65 per cent. of the maximum possible, terminal symptoms appear; these are decreased sugar tolerance, and nervous symptoms, in pigeons opisthotonus; changes in temperature also occur; there is usually lack of vision as also bradycardia; excitement and sudden noises aggravate the symptoms. Sometimes oedema occurs. Cure takes place very rapidly upon giving the vitamin; too quickly for gross histological change to have occurred. With larger amounts of food the symptoms are induced more

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rapidly. It will be realized that symptoms of vitamin B_1 deficiency are mixed up with those of general inanition, but it is possible now to separate the two. The problem has been to find out which symptoms are primary, so that we may be able to view the question in proper perspective.

Some experimental facts established recently in the Oxford Department of Biochemistry about vitamin B_1 seem to be of so much interest that they should be widely known by those whose work brings them into contact with beriberi or vitamin B_1 deficiency. Briefly, it is now clear that vitamin B_1 is intimately concerned with the degradation by oxidation or otherwise of the substance pyruvic acid. The experimental work provides a proof from another angle that this acid is an important intermediary of carbohydrate metabolism; this possibility had been suspected from other indirect evidence based upon experiments performed elsewhere with tissues *in vitro* under rather artificial conditions of hydrogen ion concentration and by the use of tissue poisons. In absence of the vitamin there is an accumulation of pyruvic acid owing to the stoppage of metabolism at this stage. This induces a biochemical lesion in some tissue cells, and therefore their activity is inhibited. These facts are clear: they provide probably an explanation of some of the other more obscure symptoms found in the vitamin B_1 deficient animal.

Historical.

It has long been suggested that vitamin B_1 was concerned with carbohydrate metabolism. We may trace this idea at least from 1912 onwards, especially in the writings of Funk (1914, 1922) and of Braddon and Cooper (1914), and later in that of the French worker Randoin (1923). The evidence supporting this view was at best indirect; large amounts of food were shown to cause the appearance of avitaminous symptoms more quickly, but this is generally summed up in the statement that finally found expression in Cowgill's formula:

$$\frac{\text{Vitamin } B_1}{\text{Calories}} = k \times \text{weight.}$$

A close examination of the earlier work, which purported to support the idea that there are changes in intermediary carbohydrate metabolism in vitamin B_1 deficiency carried no conviction except in two respects, *viz.*, the finding that blood lactic acid was raised (first noticed by Collazo and Morelli, 1925) and that there was decreased tolerance to sugar in the terminal stages. Even the increased blood lactic acid was not found to be a constant phenomenon. Similarly, the view that changes in tissue oxidation were present and responsible for the symptoms received no universal support; the evidence for this is to be traced back to Dutcher (1918) and Findlay (1921), and it was energetically investigated by Abderhalden and Wertheimer (1920) and Hess (1921). The work however carried no real conviction, and it is now clear that several statements were not correct. Also there was not complete control of

the question of inanition, as DRUMMOND and his colleagues originally showed in 1926/1927. An especially exhaustive account of experiments upon tissue oxidation was published by RYDIN (1935); here he showed that the claim could not be substantiated that there was any change in tissue oxidation, as investigated by dyes such as methylene blue. The more recent work has succeeded because of the realization that the brain tissue held the key to this problem.

It had been noticed by HESS in 1921 that pigeons suffering from cyanide poisoning tended to have symptoms similar to those of the so-called polyneuritic* or beriberi pigeon. I found in 1927 that an overdose of insulin in pigeons also



Opisthotonus symptoms in pigeon produced by insulin.

induced a similar condition, a picture of this I reproduce here. (It may be noticed incidentally that birds tolerate enormous doses of insulin.) This led Mr. KINNERSLEY and myself (KINNERSLEY and PETERS, 1929, 1930) to investigate the intermediary carbohydrate metabolism in these brains. Brain tissue *in vitro* is known, as do other cells, to induce a rapid glycolysis; *i.e.* the degradation of glucose ——> lactic acid (CH_3.CHOHCOOH). We found this effect to be unaltered in the brain brei from the avitaminous animal. On the other hand, there was abnormality in lactic acid metabolism. The brain tissue from pigeons

* I consider this term a bad one, now that there is so much evidence that the symptoms are central in origin.

suffering from opisthotonus was found to contain larger amounts of lactic acid than the normal. We found, as McGINTY and GESELL (1925) had done, that it was necessary to work very quickly to show any of these effects. Brains had to be removed with the guillotine, and plunged rapidly into liquid air. The upshot was to confirm the idea that the seat of the trouble lay in the brain, and to suggest to us that the first phases were to be found in the lower parts of the brain. Curiously enough we have never been able to convict the cerebellum of abnormality!

The next step was to investigate again, as others had done, the oxygen uptake in suitable apparatus *in vitro* in buffered Ringer's solutions; but we made the change that after mashing the brain tissue, the samples were suspended in Ringer-phosphate (pH 7·3) with additions of lactic acid or glucose. Without the latter substances, Dr. GAVRILESCU and I could find nothing certainly abnormal in the uptake (GAVRILESCU and PETERS, 1931); but when these were added, there was noticeable a definitely smaller respiration than the normal. Further, the most interesting point of all was that vitamin B_1 itself in very small amount 0·001 mg./3 c.c. restored the respiration of the brain mash almost

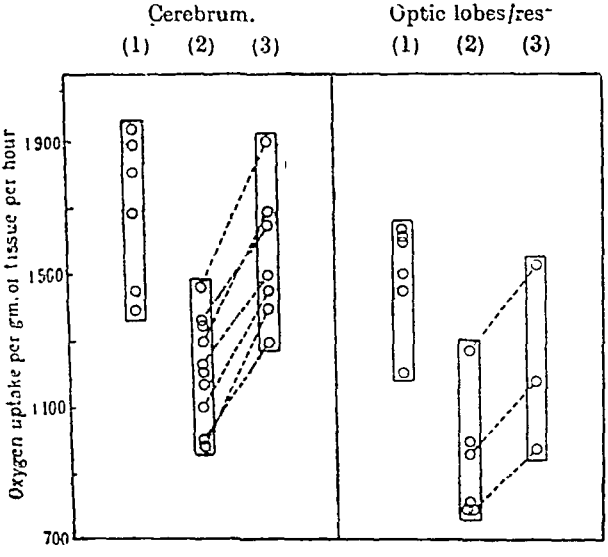


FIG. 1.—Oxygen uptake $mm^3/g/hr.$ in presence of 0·037 M lactate, for (1) normal. (2) avitaminous and (3) avitaminous brain with addition of vitamin B_1 concentrates. Pigeon, From *Proc. R. Soc.* (1932). Ser. B, 110, 439.

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though vitamin B_1 restored respiration when lactate was present in the medium, there was no increased disappearance of the lactate. Hence some further intermediate of oxidation was involved. Search has proved that this was pyruvic acid ($CH_3.CO.COOH$), the natural step for oxidation of lactate. H. M. SINCLAIR and the author were led to look for this in the bottles in which the pigeon's brain had been respiring (PETERS and SINCLAIR, 1933).* There is a convenient reaction of SIMON and PIAUX for pyruvic acid, resembling in technique that for the Rothera; one saturates a small amount of the solution (1 to 2 c.c.) with ammonium sulphate until a layer of crystals remains at the bottom of the test tube; upon this is placed a diffused layer of sodium nitroprusside crystals, and then an equal volume of concentrated ammonia is cautiously poured on top. Pyruvic acid is detected by the presence of a slowly developing blue green colour. We know now from the work of JOHNSON and EDWARDS (1937), that this reaction is given by urine of men shortly after running to exhaustion.† We found that it was present in the bottles containing no vitamin, but absent when vitamin had been present. The question was studied further by R. H. S. THOMPSON and the author quantitatively (PETERS and THOMPSON, 1934). The substance giving the blue green reaction was identified as pyruvic acid chemically (formation of the 2:6 dinitrophenylhydrazone), and quantitative work gave results of the kind given in the accompanying table.

TABLE.

DISAPPEARANCE OF PYRUVATE AND INCREASE IN O_2 UPTAKE BY THE ACTION OF VITAMIN B_1 WITH AVITAMINOUS PIGEON BRAIN RESPIRING *in vitro*. SUBSTRATE LACTATE. AVERAGE OF SIX EXPERIMENTS. FIGURES MG./GM. PYRUVATE PRESENT AT END OF 2-HOUR PERIOD OF RESPIRATION.

L.	L + V.	Difference.	Difference O_2 uptake mm/^{3}gm.
3·40	1·66	1·73	+ 1,102

Period of respiration = 2 hours. L = lactate. L + V = lactate + vitamin.
(From results of PETERS and THOMPSON, 1934.)

* We were led to search for this acid because of the prevailing interest at the time of the EMBDEN-MEYERHOF scheme of muscle fermentation. According to this, sugar is broken down by a complicated series of phosphorylations in which pyruvic acid plays an important rôle.

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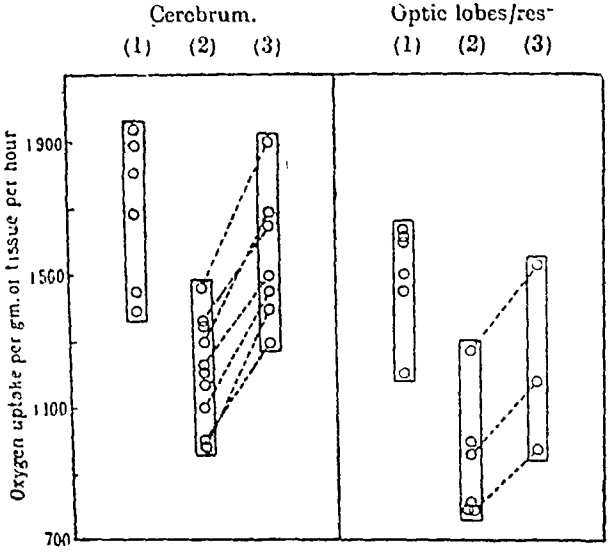


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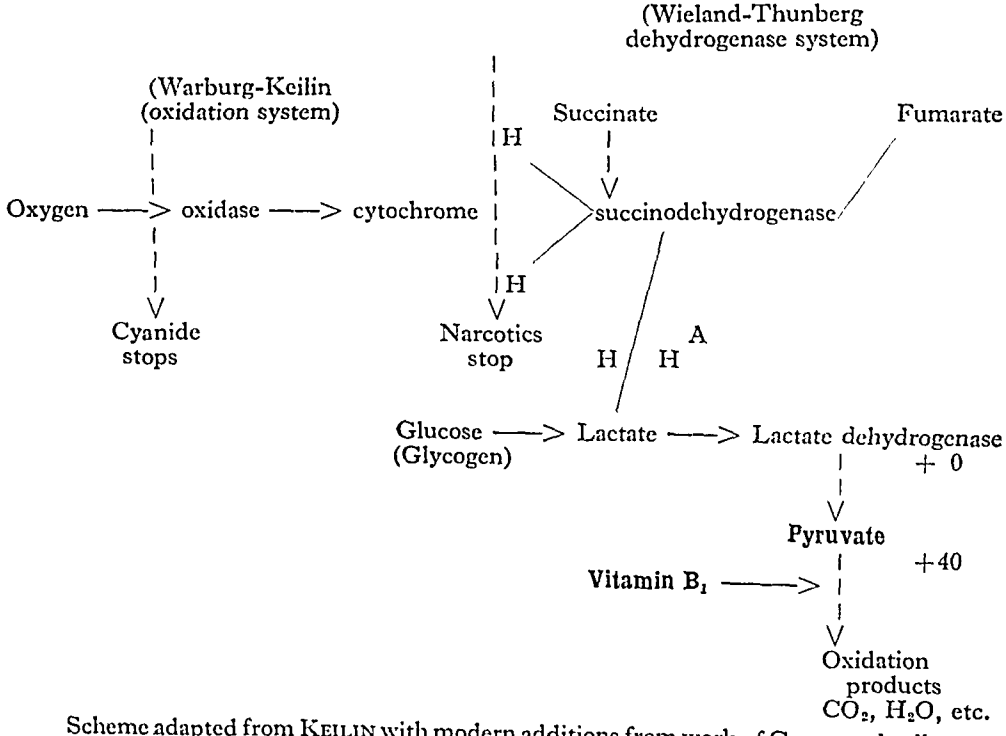
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Hence there was now proof that the appearance and disappearance of the organic substance pyruvic acid was regulated by the amount of vitamin present. I have purposely left out one essential part of the proof until the present time for clarity. It might be said that there was a general failure of tissue oxidation in the avitaminous brain rather than a specific one. That this is not so was first shown clearly by the use of succinate ($COOH.CH_2.CH_2.COOH$). This is a four-carbon atom compound present in muscle and other tissues which is actively metabolized by tissue *in vitro*; but its oxidation is quite normal even in the abnormal brain, and there is no influence of vitamin upon it. So it is with many other possible substances tried. We may now embody this and certain other results in the following diagrams. Fig. 2 is adapted from KEILIN and WARBURG; it shows the place of this oxidation in the scheme of tissue respiration by which oxygen is activated by the Warburg-Keilin oxidation system to be handed on to the hydrogen activated by the dehydrogenases. There is indicated specifically in black type what happens with lactic and pyruvic acid in relation to the point of action of the vitamin.

FIGURE 2.

SCHEME OF TISSUE OXIDATION.



Scheme adapted from KEILIN with modern additions from work of GREEN and colleagues (at Cambridge). Hydrogen is mobilized by the specific dehydrogenase systems, ultimately to reduce oxidized cytochrome. Transfer of H at A is speculative; it is possible that pyruvate is first formed from glucose and then reduced to lactate.

Our next enquiry is how far this condition is really specific to the avitaminous state, and what organs of the body show it. The diagram from MEIKLEJOHN, PASSMORE and PETERS (1932) shows that as the bird becomes cured by

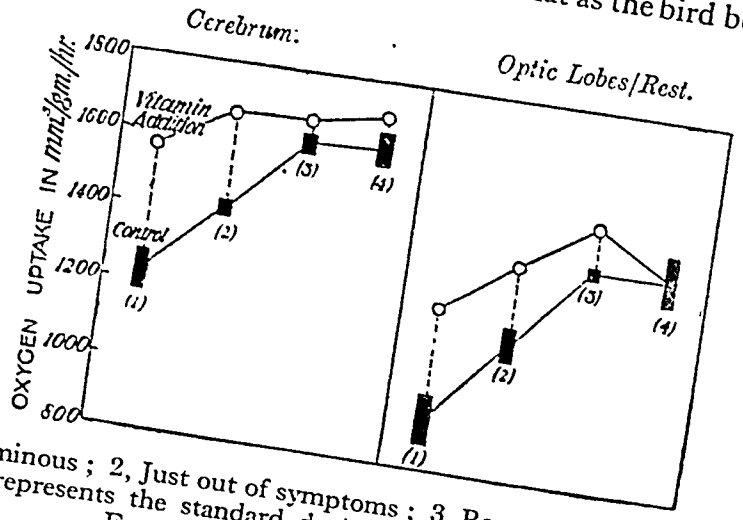


FIG. 2.—1, Avitaminous; 2, Just out of symptoms; 3, Recovered well; 4, Normal. The black rectangles represents the standard deviation of the mean of the observations. From *Proc. R. Soc.* (1932). Ser. B., 3, 394.

vitamin B_1 (and this is a rapid process), so abnormality of the respiration decreases together with the corresponding effects of vitamin upon the system. With normal brain, the action of vitamin is only slight if at all present. This contrasts with the large effects observed in the avitaminous brain, and forms a very important link between the brain enzyme systems which we found *in vitro* and the condition of the brain in life.

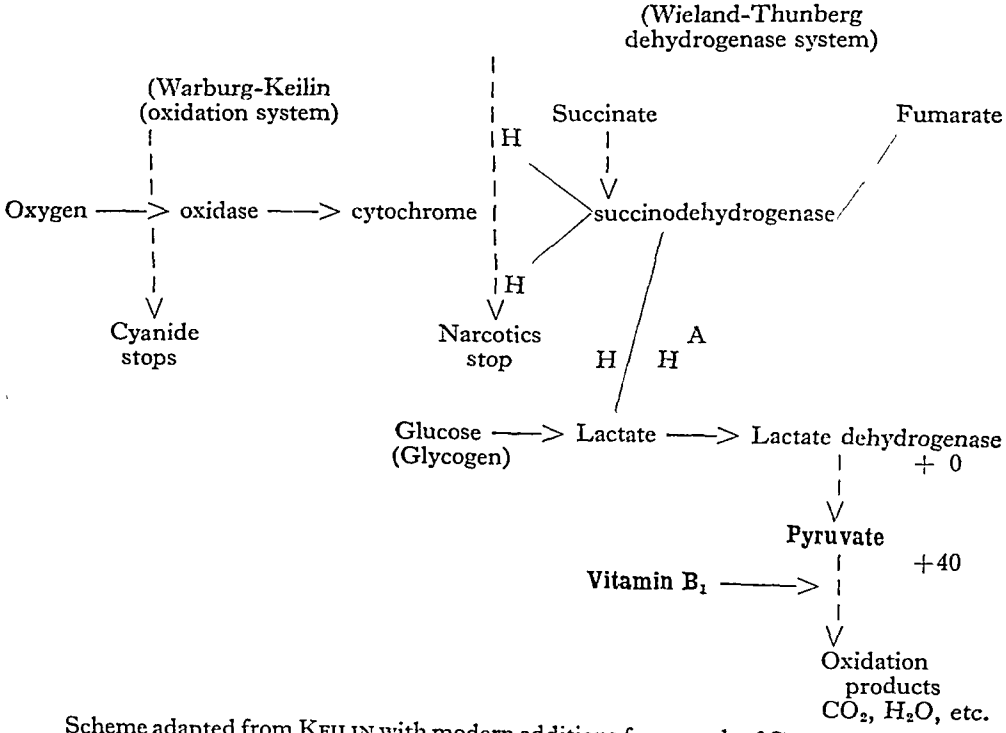
In the case of other parts of the avitaminous bird, THOMPSON (1934) was only able to find a catatorulin effect with the kidney. This is interesting in relation to the latent oedema in these birds, which can be developed by giving them salt. An effect has been reported by SHERMAN and ELVEHJEM (1936*a*) for the heart, but this is not so clear. They have also confirmed these results for chicken brain (1936*b*), and rather similar effects have been obtained by O'BRIEN and PETERS (1935) for the rat brain. The facts seem therefore to be general.

We now come to the important point discovered by THOMPSON and investigated by himself and JOHNSON (THOMPSON and JOHNSON, 1935), namely that pyruvic acid is abnormally present in the blood of the avitaminous pigeon and rat up to 10 mg. per 100 c.c. blood; it disappears upon cure of the animal. This can be estimated by use of the property of pyruvic acid to bind bisulphite, a property which it shares with other keto- and aldehyde substances. In the case of the animal, the increase in B.B.S. value has been found by JOHNSON to be mainly due to pyruvic acid. (In man, PLATT has stated that this is not so.) Hence, we have been led to find a biochemical abnormality in the blood by a

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FIGURE 2.

SCHEME OF TISSUE OXIDATION.



Scheme adapted from KEILIN with modern additions from work of GREEN and colleagues (at Cambridge). Hydrogen is mobilized by the specific dehydrogenase systems, ultimately to reduce oxidized cytochrome. Transfer of H at A is speculative; it is possible that pyruvate is first formed from glucose and then reduced to lactate.

Our next enquiry is how far this condition is really specific to the avitaminous state, and what organs of the body show it. The diagram from MEIKLEJOHN, PASSMORE and PETERS (1932) shows that as the bird becomes cured by

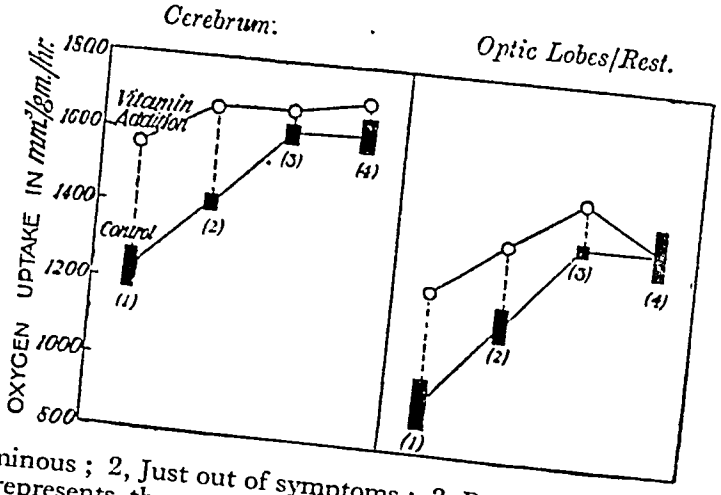


FIG. 2.—1, Avitaminous; 2, Just out of symptoms; 3, Recovered well; 4, Normal. The black rectangles represents the standard deviation of the mean of the observations. From *Proc. R. Soc.* (1932). Ser. B., 3, 394.

vitamin B_1 (and this is a rapid process), so abnormality of the respiration decreases together with the corresponding effects of vitamin upon the system. With normal brain, the action of vitamin is only slight if at all present. This contrasts with the large effects observed in the avitaminous brain, and forms a very important link between the brain enzyme systems which we found *in vitro* and the condition of the brain in life.

In the case of other parts of the avitaminous bird, THOMPSON (1934) was only able to find a catatorulin effect with the kidney. This is interesting in relation to the latent oedema in these birds, which can be developed by giving them salt. An effect has been reported by SHERMAN and ELVEHJEM (1936*a*) for the heart, but this is not so clear. They have also confirmed these results for chicken brain (1936*b*), and rather similar effects have been obtained by O'BRIEN and PETERS (1935) for the rat brain. The facts seem therefore to be general.

We now come to the important point discovered by THOMPSON and investigated by himself and JOHNSON (THOMPSON and JOHNSON, 1935), namely that pyruvic acid is abnormally present in the blood of the avitaminous pigeon and rat up to 10 mg. per 100 c.c. blood; it disappears upon cure of the animal. This can be estimated by use of the property of pyruvic acid to bind bisulphite, a property which it shares with other keto- and aldehyde substances. In the case of the animal, the increase in B.B.S. value has been found by JOHNSON to be mainly due to pyruvic acid. (In man, PLATT has stated that this is not so.) Hence, we have been led to find a biochemical abnormality in the blood by a

research which had its origin in "*in vitro*" experiments of an academic type upon brain enzyme systems—an interesting instance of how research works out. It should be noted that it is a mistake to think that the pyruvic acid itself is responsible for the symptoms. It is only a reflection of tissue abnormality; DE JONG (1936), in Holland, with a beautiful microtechnique found cases in pigeons in which the opisthotonus did not run parallel with the change in the blood B.B.S. value. Further, there is no evidence that injections of pyruvic acid are toxic. The symptoms are due to dysfunction of certain cells in the brain due to the failure of oxidation at the pyruvic acid stage.

The brain lesion has been further followed by MCGOWAN (1937) in the attempt to investigate the oxidation of the pyruvic acid. This question is somewhat outside the scope of this article; quantitative work shows that 3/5 to 4/5 of the pyruvic acid is completely oxidized. The fate of the remainder is unknown. It is not believed to pass through succinic acid, though this has been proved to be an intermediary in anaerobic brain metabolism (WEIL-MALHERBE, 1937). Attempts have been made also to trace the exact way in which vitamin B_1 exerts its effect upon pyruvic acid. Here a very recent discovery by LOHMANN and SCHUSTER (1937*a*, *b*) is of outstanding importance and interest. It means no less than that a vitamin B_1 compound is an important catalyst, *viz.* (co-carboxylase), in the formation of alcohol by yeast, and so in alcoholic fermentation; the compound is a vitamin B_1-pyrophosphoric ester. The reaction catalysed by the carboxylase enzyme is $CH_3.CO.COOH \longrightarrow CH_3CHO + CO_2$, the aldehyde being subsequently converted to alcohol. In the microorganism there is therefore also evidence that vitamin B_1 is concerned with pyruvic acid. Whether the vitamin B_1 pyrophosphate is present in brain tissue has yet to be proved The *in vitro* effect is better exerted by vitamin B_1 itself, in our experiments.

Explaining the symptoms as due to an enzyme fault (failure in pyruvate oxidase) we are still left with certain other abnormalities, namely (1) the initial failure in appetite, which occurs long before any other avitaminous manifestations. This has been much studied by COWGILL and colleagues. (2) There is also the increase in blood lactic acid and the change in the sugar tolerance in the terminal stages of deficiency together with possible disturbances in the regulation of glycogen stored in the liver. (3) Also bradycardia* (DRURY, HARRIS and MAUDSLEY, 1930) and (4) Tendency to oedema. No. 2 can be brought into line by the following hypothesis; the lactic acid accumulates (see Fig. 2) because there is a block in the further oxidation of lactic acid by pyruvate. This is known to happen in isolated enzyme systems from the work of GREEN and BROSTEAUX (1936). The changes in sugar tolerance, etc., may well be due to the fault in brain cells, caused by the enzyme failure. It is not unreasonable to suppose that there is interference with the central regulation of sugar metabolism; on this view the initial enzyme fault in intermediary carbohydrate metabolism induces a secondary change in carbohydrate regulation. To the fault in the brain may also be ascribed certain secondary changes such as interference with temperature regulation. No. 4, the tendency to oedema, can be explained along

* Not to be confused with the dietary heart block of pigeons (CARTER and DRURY) which is not due to lack of vitamin B_1, but some other factor.

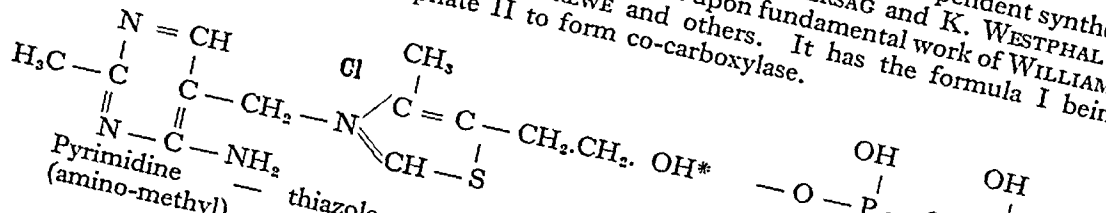
similar lines by the fault found in the kidney cells. The bradycardia (
yet been explained properly, and as yet we have no clear ideas as to
failure of appetite is induced. If it is caused reflexly by some diminuti
concentration of this vitamin in the blood some cells must be sensitive
minute changes in concentration of this substance, which is almost inc
though not impossible in living matter. That changes in the blood vita
concentration probably occur is indicated by work of MEIKLEJOHN (1937
the use of a modified mould test of Schopfer in which the growth of the
organism (*Phycomyces blakesleeanus*) with vitamin as catalyst is used as a
much lowered in the deficient condition. Similarly in unpublished work H.
SINCLAIR considers that there can be variations in human blood in disease ;
amount present in the normal human appears to be about 0·008 mg./100
blood.

Much of the above experimental work upon animals has now been foun
by Dr. PLATT and his colleagues in excellent work to apply to the human. S
that we can have confidence in the practical application of studies of anima
research in this way.

APPENDIX.*

Some Chemical Points.

Vitamin B_1 chloride, hydrochloride is now available owing to the independent synthesis by R. R. WILLIAMS and J. K. CLINE in America, by H. ANDERSAG and K. WESTPHAL in Germany, and BERGEL and TODD in this country based upon fundamental work of WILLIAMS, CLARKE and colleagues, WINDAUS and GREWE and others. It has the formula I being substituted at * with pyrophosphate II to form co-carboxylase.



Pyrimidine (amino-methyl) — thiazole (hydroxy ethyl) I

II

About 0·5 to 1·0 mg. is the daily dose for man, and about 0·005 to 0·02 mg. for a small animal. It can be detected chemically either by a special azo test (KINNERSLEY and PETERS), or by conversion to a blue fluorescent oxidation product (thiochrome of Kuhn) by oxidation with potassium ferricyanide in alkaline solution. The blue fluorescence is detectable in the ultra-violet light. Pyruvic acid can be estimated either by use of its property of binding bisulphite or by conversion to the 2-4 dinitrophenylhydrazone ; the latter forms a strong reddish colour in alkaline alcoholic potash solution which can be estimated colorimetrically. This is more specific. The bisulphite binding property is shared with substances like aceto-acetic acid, which must therefore be excluded if this is used.

* For references see *Ann. Rev. Biochem.*, 1937 and 1938 (in Press), articles on water-soluble vitamins.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

BIOCHEMICAL CHANGES, ESPECIALLY OF PYRUVIC ACID, IN RELATION TO SOME CLINICAL FEATURES OF BERIBERI.

BY

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Recent investigations have led to considerable advances in our knowledge of diseases due to the deficiency in the body of essential food factors. A good illustration is afforded by the progress made in the study of vitamin B_1 in its relationship to beriberi.

In common with other deficiency states, developments have followed rapidly on the identification and preparation of the pure vitamin. The effects of pure vitamin B_1 on tissues have now been investigated and, by appropriate biochemical methods, well-defined changes have been discovered in tissues from experimental animals deprived of the vitamin.

It is evident from the results of PETERS and his co-workers (PETERS and SINCLAIR, 1933; PETERS and THOMPSON, 1934, and THOMPSON and JOHNSON, 1935), that pyruvic acid is of importance in relation to the metabolic processes in vitamin B_1 deficiency states. It therefore appeared probable that information might be obtained from an investigation of the changes in pyruvic acid in the body fluids of patients suffering from beriberi. Blood, cerebrospinal fluid and urine have now been examined for the occurrence of pyruvic acid.

Since quantities of blood and cerebrospinal fluid were being withdrawn as a therapeutic measure, it was possible to isolate and identify pyruvic acid as the 2 : 4 dinitro-phenylhydrazone from both these fluids as well as from urine (PLATT and LU, 1935 and 1936). It is of interest to note that occurrence of pyruvic acid in the blood of vitamin B_1 deficient animals was established by similar methods at about the same time by JOHNSON (1935).

For the detection and estimation of pyruvic acid in smaller amounts of body fluids, three methods have been employed. The first was a method based on the extraction of the 2 : 4 dinitro-phenylhydrazone. This method appeared to be sufficiently precise and specific for pyruvic acid so that it has been employed extensively and is the method by which most of the results reported in this communication were obtained. The second method depends on the property which pyruvic acid possesses in common with other carbonyl compounds of binding bisulphite. The total bisulphite binding substances (B.B.S.) however are expressed in terms of pyruvic acid (mg. per 100 gramme of fluid). A third test, the nitroprusside reaction, is believed to be specific for pyruvic acid but cannot be used for accurate quantitative determinations. It is, however, of value where facilities do not exist for applying the hydrazone method.

The results of estimations of total B.B.S. are of interest (Editorial, *Lancet*, 1938) and may be presented at this point. Various figures have been reported for the amounts in the blood of apparently normal subjects; a summary of the results is given in the table on page 495.

The differences between these figures are probably accounted for by the condition of the subjects when blood was taken for examination.

Three or four times these amounts of B.B.S. were reported by PLATT and LU (1935) in cases of fulminating beriberi, the values ranging from 7 to 16 mg. per cent. (PLATT and LU, 1936). WILSON and GHOSH (1937) found a mean value in epidemic dropsy of 8·33 mg. per cent. for blood.

It must, however, be recognized that elevation of B.B.S. may occur in conditions other than vitamin B_1 deficiency. WILKINS *et al.* (1937) have observed increases in conditions associated with acidosis, ketosis, anoxaemia, "toxaemia" and uraemia.

Furthermore, even in beriberi, usually not more than about half of the increase in B.B.S. was accounted for by increase of pyruvic acid, when this was estimated by the hydrazone method (PLATT and LU, 1936). However, pyruvic

acid values and total B.B.S. fall to normal levels in such cases of beriberi when adequate amounts of vitamin B_1 are administered.

TABLE.

Investigators.	Country.	Results.
JOHNSON, *et al.* (1935)	England	22 subjects. Range 1·96-4·0 mg. per 100 gm. blood. Average 2·81.
PLATT and LU (1936)	China	23 subjects. Range 2·22-4·82 mg. per 100 gm. blood. Mean 3·27, standard deviation 0·70.
WILKINS, *et al.* (1937)	U.S.A.	30 subjects. Range 3·66-5·75 mg. per 100 ml. Average 4·74.
WILSON and GHOSH (1937)	India	6 subjects. Range 2·9-3·77 mg. per cent. Average 3·38.

These observations in man differ from those recorded in experimental vitamin B_1 deficiency in animals. For it has been shown (THOMPSON and JOHNSON, 1935) that the additional B.B.S. accumulated in the blood of the rat and pigeon in vitamin B_1 deficiency are almost wholly accounted for by increase in pyruvic acid as determined by the hydrazone method.

Attention may be directed to the observation that the pyruvic acid level may be raised, whilst the B.B.SS. are within the normal range. This is now known to be true of some of the patients whose B.B.S. values have been recorded as normal (PLATT and LU, 1936, Table III) whilst the pyruvic acid values range from 0·41 to 1·27 mg. per cent. These results may be taken as further evidence in support of the suggestion previously made (PLATT and LU, 1936) that pyruvic acid should be sought for in all cases of vitamin B_1 deficiency.

In view of the observations of PI SUÑER and FARRÁN (1936) that pyruvic acid occurs in the urine of compensated diabetic patients, it may be that increased amounts of pyruvic acid in body fluids are not necessarily always associated with vitamin B_1 deficiency. This is a matter for further investigation. It would, however, not be surprising to find abnormal amounts of pyruvic acid in the body in conditions in which disturbances of intermediate carbohydrate metabolism are involved.

The values for pyruvic acid determined by the hydrazone method for blood obtained from apparently healthy subjects or from patients who have received adequate doses of vitamin B_1 were usually between 0·4 and 0·6 mg. per 100 grammes. About half these amounts of pyruvic acid were present in cerebro-

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spinal fluid from similar subjects. It is important as will be seen later to draw the blood from the subject under basal conditions.

The results of JOHNSON *et al.* (1935) indicated that normal blood contained less than 1·64 mg. per cent. Later, JOHNSON and EDWARDS (1937) reported values of 0·1 to 0·18 mm. per litre for blood (*i.e.* 0·66 to 1·49 mg. per cent.).

The blood of the fulminating type of beriberi contained more than 1 mg. per cent. and values as high as 6 and 7 mg. per cent. have been recorded. The values for pyruvic acid in the blood of a series of usually fatal cases of this acute form of vitamin B_1 deficiency have been published (*Annual Report, 1936*). Of twenty-one cases, seven individuals had values between 1 and 2 mg. per cent., and the values of the remaining fourteen ranged from 2 to 4·5 mg. per cent. Seventeen of these patients were cured with a single dose of pure vitamin given intravenously. Four died within a short time after administration of a similar dose ; three of the four fatal cases had pyruvic acid values over 2 mg. per cent.

The amount of vitamin found to be effective in the cure of the fulminating cases was 5 mg. The injection was always given intravenously as a solution made up from the crystalline substance in acid-alcohol. Products from natural and synthetic sources appeared to be equally effective.

When no complicating factors were present, the pyruvic acid in the blood fell steadily to the normal level in the course of 10 to 15 hours, after a dose of vitamin B_1. The patients who did not respond to treatment died before normal levels had been attained.

A number of patients who had some febrile disease associated with evidences of vitamin B_1 deficiency also failed to respond to treatment with vitamin B_1. These individuals usually died within a few hours after treatment. Generally, the accumulation of pyruvic acid in the blood was not so marked in these as in the cases uncomplicated by other disease. However, if a remission of the fever occurred, the prominent symptoms of vitamin B_1 deficiency could be relieved by injection of vitamin B_1 and the pyruvic acid level was found to return to normal. With recurrence of fever a marked rise of pyruvic acid occurred and some of the more acute symptoms of vitamin B_1 deficiency also developed again.

If the blood pyruvic acid levels of a " cured " case of fulminating beriberi were determined daily after treatment with a single dose of 5 mg. of vitamin, it was usual to find that a rise occurred, even with resting subjects, about the fifth day. The increase was maintained until a further dose (2 to 3 mg.) was given. The explanation of this phenomenon was found to be related to the diet.

Immediately after recovery from the acute phase of beriberi, the patient was allowed to take the customary diet. About three-quarters of the calorie value of a typical diet was obtained from rice. The rice taken by the patients under consideration was a highly milled product and, in the amounts taken per day, supplied about 0·3 mg. of vitamin B_1. The estimated daily intake of the average patient who develops beriberi is about 0·5 mg. per day. After a single

dose of 5 mg. of vitamin B_1 measurements of food intake show that the appetite temporarily improved and the calorie intake in the form of rice doubled in the course of 2 or 3 days. For the metabolism of this increased amount of carbohydrate in the diet, more vitamin B_1 was required than was furnished by the rice. In consequence, the metabolism of cells for which vitamin B_1 is essential was deranged and one of the products of metabolism—pyruvic acid—was found to accumulate in the blood. Increased intake of carbohydrates therefore, as well as fever, appears to influence metabolic processes in the body in vitamin B_1 deficiency states.

Increased metabolic activity arising as a result of muscular effort may also be expected to accentuate the disturbances found in vitamin B_1 deficiency. Experiments were therefore made to determine the effect of exercise on blood pyruvic acid in the normal and in the vitamin B_1 deficient subject.

Examination of a " normal " individual showed that immediately after mild exercise a rise of pyruvic acid occurred in the blood, but the pyruvic acid value rapidly returned to normal. Mild exercise of a subject in a state of vitamin B_1 deficiency having a normal or slightly raised resting pyruvic acid level produced an increase of pyruvic acid in the blood immediately after exercise. In this type of subject the raised value was found to be maintained for a comparatively long period.

Severe muscular effort in a normal subject has been shown (JOHNSON and EDWARDS, 1937) to be accompanied by a marked rise in the pyruvic acid level in the blood (3 to 4 mg. per cent.). Such an accumulation of pyruvic is comparable with that observed in fulminating beriberi. In less than an hour, however, the pyruvic acid returned to its resting value.

When a " cured " case of beriberi who has been kept for some time after treatment with a single dose of vitamin on his customary vitamin B_1 deficient diet is subjected to severe exercise and considerable increase in blood pyruvic acid induced, the recovery phase after exercise was found to be 3 to 4 hours. Moreover, the subject became exhausted after an amount of exercise insufficient to cause any distress in a healthy normal subject.

The foregoing observations demonstrate three main points : firstly, that there are metabolic disturbances arising in consequence of vitamin B_1 deficiency as evidenced by accumulation of pyruvic acid in the blood ; secondly, a connection with vitamin B_1 is clear from the fact that administration of an adequate amount of pure vitamin B_1 is followed by a restoration of pyruvic acid to normal levels ; thirdly, factors which are known to intensify metabolism in the body—fever, increased food intake and muscular work—accentuate the accumulation of pyruvic acid in the body in vitamin B_1 deficiency.

It may therefore be argued from these observations that factors which affect metabolism are likely to play a part in the development of the phenomena of beriberi.

The metabolic disturbances are most marked in the fulminating type of beriberi and it is in this group that accentuating circumstances appear definitely to contribute to the onset of the acute phase. The condition commonly occurs in active, usually muscular subjects who have continued to work until shortly before the attack. The acute phase is characteristically of short duration. It is remarkable that this form is rarely seen except in hot and humid weather when sustained muscular effort is difficult and proper rest and sleep often impossible.

The operation of factors other than the degree of vitamin B_1 deficiency in the development of the various types of beriberi appears to be so important that it is a question whether considerable differences in the magnitude of the vitamin defect are likely to arise.

In this connection it is important to point out that the vitamin B_1 content of the daily diets in areas endemic for beriberi is about 0·5 mg. per person. Under ordinary conditions diets completely free from vitamin are not taken and an intake of 0·75 mg. daily represents a " border-line " supply. WILLIAMS (1938) suggests that for optimal nutrition with respect to vitamin B_1 it may be necessary to allow as much as 4 mg. per day. Yet, the foregoing results show that it is possible to " cure " a case of fulminating beriberi with a 5 mg. dose of vitamin B_1. Furthermore, there is evidence that the body does not store appreciable amounts of vitamin B_1.

In contrast with the fulminating type of beriberi, the development of the " dry, atrophic " type may be the result of a similar grade of vitamin insufficiency to which the patient has been subjected for some time in the absence of appreciable accentuating factors. The view may be advanced that nervous tissue is attacked primarily because of all the body tissues its need for vitamin B_1 for normal metabolism is most pronounced. Moreover, when nervous tissue is predominantly involved, the localisation of the disturbances appears to be determined by metabolic factors especially related to work. No significant alterations of blood pyruvic acid are found in this type of beriberi (PLATT and LU, 1936).

The incidence in hospital practice in Shanghai of these two extreme types of beriberi in their well-developed forms is only about 10 per cent. of the total cases presenting manifestations of vitamin B_1 deficiency. The majority of the cases cannot be clearly classified.

On clinical grounds, accentuating factors relating to metabolic activity in the body appear to play a part in the occurrence of these less severe forms of beriberi. For example, it was pointed out (PLATT and GIN, 1934) that nearly half of a hundred patients traced the onset of their symptoms of beriberi to a short bout of fever.

Further discussion of the features of these cases is not possible in this short communication. It is, however, important to recognize the part played in the symptomatology of these cases by changes which are secondary to impair-

ment of the function of tissues and organs and that the clinical picture is often complicated by association with other disease.

Nevertheless, noteworthy improvement may follow treatment with amounts of vitamin B_1 of the same order as have been used for the fulminating cases. There may, however, be no remarkable variations in the levels of pyruvic acid in the blood. In some instances, disturbances appear to have progressed to the stage at which recovery cannot be effected merely by supplying adequate amounts of vitamin B_1.

Summary and Conclusions.

There are substances which bind bisulphite normally present in the blood of human subjects. These substances are found in increased amounts in the blood of cases of fulminating beriberi.

Pyruvic acid is one of these substances. Increased amounts of pyruvic acid are shown to occur in trichloracetic acid extracts of blood, urine and cerebro-spinal fluid taken from patients with fulminating beriberi.

In uncomplicated cases of fulminating beriberi showing increased amounts of pyruvic acid, normal levels are restored in the course of 10 to 15 hours after intravenous administration of 5 mg. of pure vitamin B_1.

Vitamin B_1 has been shown by other workers to be concerned in the intermediate metabolism of carbohydrates. Changes in amounts of metabolites in the blood in vitamin B_1 deficiency states are considered to be evidences of disturbances arising in tissues whose carbohydrate metabolism involved vitamin B_1.

The operation of some factors—fever, increased intake of carbohydrates, muscular effort—which are known to affect metabolism in the body, leads to accumulation of pyruvic acid in the blood in vitamin B_1 deficiency states.

It is suggested that the effects of accentuating factors contribute to the development of the various clinical types of beriberi. These effects may be of more importance in this respect than the differences in the grade of vitamin B_1 deficiency. When accentuating factors are minimal it is probable that well-defined changes only appear after a comparatively long period of deficiency.

Changes secondary to the failure of various organs and associated disease modify the clinical picture of beriberi.

The effects of treatment with vitamin B_1 must be assessed with cognisance of the fact that damage to tissues may ensue, especially in long term deficiency, which cannot be repaired merely by correcting the vitamin deficiency.

The metabolic disturbances are most marked in the fulminating type of beriberi and it is in this group that accentuating circumstances appear definitely to contribute to the onset of the acute phase. The condition commonly occurs in active, usually muscular subjects who have continued to work until shortly before the attack. The acute phase is characteristically of short duration. It is remarkable that this form is rarely seen except in hot and humid weather when sustained muscular effort is difficult and proper rest and sleep often impossible.

The operation of factors other than the degree of vitamin B_1 deficiency in the development of the various types of beriberi appears to be so important that it is a question whether considerable differences in the magnitude of the vitamin defect are likely to arise.

In this connection it is important to point out that the vitamin B_1 content of the daily diets in areas endemic for beriberi is about 0·5 mg. per person. Under ordinary conditions diets completely free from vitamin are not taken and an intake of 0·75 mg. daily represents a " border-line " supply. WILLIAMS (1938) suggests that for optimal nutrition with respect to vitamin B_1 it may be necessary to allow as much as 4 mg. per day. Yet, the foregoing results show that it is possible to " cure " a case of fulminating beriberi with a 5 mg. dose of vitamin B_1. Furthermore, there is evidence that the body does not store appreciable amounts of vitamin B_1.

In contrast with the fulminating type of beriberi, the development of the " dry, atrophic " type may be the result of a similar grade of vitamin insufficiency to which the patient has been subjected for some time in the absence of appreciable accentuating factors. The view may be advanced that nervous tissue is attacked primarily because of all the body tissues its need for vitamin B_1 for normal metabolism is most pronounced. Moreover, when nervous tissue is predominantly involved, the localisation of the disturbances appears to be determined by metabolic factors especially related to work. No significant alterations of blood pyruvic acid are found in this type of beriberi (PLATT and LU, 1936).

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DISCUSSION.

Miss Harriette Chick, D.Sc.: As a laboratory worker on vitamins I would wish to offer my warm congratulations to the authors of these papers. To those of us who have been struggling with the experimental work on this subject it is wonderful to hear this story of the striking clinical effect of crystalline vitamin B_1 which is now available for the treatment of acute beriberi. There have been many disappointing and discouraging reports from workers in clinical medicine in the tropics, full of doubt as to whether vitamin B_1 had any real importance in the etiology of beriberi. The work which has been reported this evening is very encouraging, as giving evidence which is overwhelming that vitamin B_1 has *all* to do with the development and symptomatology of beriberi, anyhow in this particular form. I would like to know whether any explanation can be offered of the contradictory opinions which have been expressed in the past in the published clinical work on this subject.

Professor R. Brunel Hawes: The reaction is quantitative and therefore much of the disappointment has been due to too small doses. Sometimes the standardization of commercial products is not correct and mistakes with insufficient dosage occur with fatal results.

Miss Harriette Chick: What is the source ?

Professor R. Brunel Hawes: The extract from natural sources has been especially prepared for me by the Geneeskundig Laboratorium in Batavia-Centrum, Java. Other carefully standardized products were from Bayer's who produced a natural extract but now make synthetic products. Another synthetic product came from Switzerland.

Dr. P. Manson-Bahr: I have listened to the remarks of Professor PETERS with great attention, and especially to his observations upon the effect of vitamin B_1 upon the appetite in animals. Purely empirically, as far as the true etiology of the disease is concerned, I have for the last six months been using vitamin B_1 injections (*Benerva*-Roche 1·25 mgm. of crystalline B_1) daily in sprue. One of the chief characteristics of the advanced stages of the disease is anorexia, and in this respect it appears to have a remarkable effect in increasing the patient's desire for food. I have observed some ten cases in which this form of treatment has been carried out, but in eight of these it has been combined with the generally accepted liver injection therapy.

Whether the stomatitis of sprue is a separate entity or whether it is comparable to that observed in Addisonian anaemia, pellagra and the peculiar condition, now known to be due to B_2 deficiency, which was first observed by myself in Ceylon, by J. V. LANDOR and R. A. PALLISTER in Malaya, and later again by L. NICHOLLS in Ceylon, is an interesting point. I have noted, however, that in vitamin B_1 therapy in sprue there is a rapid improvement in the tongue and mouth in association with the stimulus to the appetite. By making these observations I do not wish to infer that I consider that the syndrome of sprue is due to a vitamin B_1 deficiency but merely to point out that this new and active product may play a part in the therapeutics of this distressing disease.

Professor Warrington Yorke: I suppose there is improvement as regards other symptoms *pari passu* with that of the stomatitis ?' If the patient recovers from sprue the stomatitis would disappear as part of this general improvement.

Dr. P. Manson-Bahr: Our sprues always do get better.

Professor Warrington Yorke: That is exactly the answer I expected.

Dr. J. Barcroft Anderson: The late Dr. ROWLANDS, about a year before he died, described the result of his work on rats, and he gave me the impression, so far as that work went, that the vitamins B were necessary to all the tissues of the kidney. Dr. ROWLANDS represented that continued deficiency of the vitamins of the germ of grain diminished the size of nerve cells and nerve fibres; reduced the unstriped muscle tissue to one-third of its bulk in the intestine of the rat, and produced degenerative changes in all the tissues of its kidney. I have never seen any allusion in medical literature to Dr. ROWLANDS' work, and I do not know if the speakers this evening have studied the effect on kidney tissue?

Dr. H. S. Stannus asked Professor PETERS whether there was any evidence, in man, of interference in the oxidation of carbohydrate in the brain tissue into the series of normal products he had described, by other means than a deficiency of vitamin B_1—whether toxic substances could thus act; also whether any other substance than B_1 played any part in the normal metabolism.

Professor R. A. Peters: I do not know what histamine cures mean. They do not occur at all constantly, and I believe they are really to be grouped with temporary cures which we often observe. When we worked with these avitaminous pigeons we early found that we had to be extremely careful not to be misled by temporary pseudo-cures. We usually induce the symptoms of vitamin deficiency in the cold, and then we bring the birds into a warm room, because in this condition they die off rather rapidly if the surrounding temperature is not about 70° F. We then find if we give water plus or minus a little sugar, that a number of birds will recover for a period of 2 or 3 days. If you had been testing for vitamin B_1 and had been giving a vitamin B_1 preparation you would be inclined to say that the cure had been effected by this. However, the symptoms usually come on again in a period varying from 1 to 3 or 4 days, and it is then found impossible to clear them up by giving further doses of water or sugar. We find that the birds are then quite safe to use for tests. If you take the view that vitamin B_1 has to be present in the brain for normal function, then you will also think that this vitamin has to be brought to the brain in the blood. A temporary interference with circulation will prevent vitamin B_1 from passing from other tissues to the brain. If a few more molecules of B_1 are brought to the brain by improving circulatory conditions the brain recovers temporarily. I do not know how far this is supported by Dr. SINCLAIR'S observations. The kidneys we know to be out of order, and very often we see in a bird, especially if salt is administered, a massive oedema, and it is interesting that these conditions clear up in the bird on injections of vitamin B_1 just as Professor HAWES has said is the case with human patients. I have seen cases of intense oedema in

the pigeon which could only be cured by injection of vitamin B_1 : oral administration produced no effect at all.

There is a question I wanted to ask Dr. PLATT, a very interesting question for us as specialists, whether acetaldehyde can be found in the blood of his cases. SHINDO* this year claimed to have isolated acetaldehyde from beriberi cases.

Dr. B. S. Platt: We have no evidence that acetaldehyde occurs in the blood of cases of fulminating beriberi. There are, however, bisulphite binding substances not yet accounted for.

Dr. T. F. Macrae: Professor HAWES told us there was some discrepancy between the alleged vitamin content of some preparations and the actual quantity found. Is it not possible that some vitamin B_1 had decomposed ? We know vitamin B_1 is not a very stable compound, and in the heat of those tropical countries it seems possible that there might have been some decomposition. The vitamin is specially unstable in alkaline solution. One other point: Professor HAWES mentioned that Dr. WILLIAMS had made available synthetic vitamin B_1. Dr. WILLIAMS is deservedly recognized to have done more to elucidate the chemical nature of vitamin B_1 than any other worker. The synthesis of vitamin B_1, however, was achieved in three countries independently and almost simultaneously—in Germany in the laboratories of the I.G. Farbenindustrie, in America by Dr. WILLIAMS and his co-workers, and in this country, where it was carried out by TODD and BERGEL first in the Department of Medical Chemistry in Edinburgh, and then in the Lister Institute.

Major-General Sir Leonard Rogers: I should like to draw attention to the remarkable similarity between the rapid clearing up of adult beriberi under vitamin B_1 injections, and the equally rapid recoveries from acute infantile beriberi in the Philippines following the oral administration of *tiki tiki*, an extract of rice polishings containing vitamin B, and to ask if the injection treatment has been tried in infantile beriberi.

Dr. William Hughes: I would like to corroborate what Professor HAWES has said about the striking effect of vitamin B_1 as seen in Malaya. Laboratory workers should have our sympathy: they turn out wonderful products and do not see the effect; but if they saw the effect of vitamin B_1 in beriberi they would be pleased with their efforts. One thing which would please them is the effect of the vitamin in infantile beriberi, because there is hardly anything more pathetic than to see a child with infantile beriberi: it has aphonia and paralysis, and the effect of vitamin B_1 in such cases is really magical.

* Editorial. *Lancet*, 1, 1938.

Professor Sir John Ledingham: Might I ask what is the immediate effect on the pneumogastric nerve ?

Dr. William Hughes: In infantile beriberi in my own experience it takes about 3 days before the child cries fairly well, that is, after 1,500 units in 3 days. Dr. HARIDAS,* of Singapore, has recently reported a number of cases and the dose of the vitamin he recommends is 500 to 2,000 units. The voice should return in 2 to 3 days and vigorous crying and voluntary movements in a week.

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I am not at all clear about Dr. MANSON-BAHR'S experiments. We have heard that vitamin B_1 greatly improves appetite. Could not the increased appetite of his cases of sprue treated with vitamin B_1 cause an alteration in their diet which would supply them with a factor which cures glossitis ?

Professor R. Brunel Hawes: There are one or two questions with regard to the effect on the kidneys. It is perfectly true that the incidence of many diseases varies in different races whose diet is different and the nephrotic syndrome appears to be more common in peoples whom we know to be short of B_1. But B_1 has not the slightest curative effect on this condition or on parenchymatous nephritis. In ordinary cases where the circulation is deranged, as it is in fulminating beriberi, you will find albumin and casts in the urine. Probably all the glands in the body, from the pituitary to the pancreas are deranged. In man there is no evidence that there is any effect on the actual brain itself. The death I do not think is a medullary death. When we were endeavouring to find out whether there was anything else required in the vitamin we used various other drugs. There was a theory that symptoms were

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Two other points I would like to comment on.

Firstly, with regard to the so-called atrophic form. I think there is good evidence for the belief that in the development of this condition there are two important factors. One is that the deficiency has persisted for a long time. The other is that there have not been present those intensifying factors which I pointed out were operative in the development of the acute symptoms.

Secondly, in connection with water retention, there is a point of some interest. With the disturbance of metabolism, there might well be changes in the water-binding powers of the cells resulting in an increase of intracellular fluid. The first appearance in the muscle tissue is observed as a hardening of

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Dr. Platt: The question was raised about infantile beriberi. In our series of fulminating cases (PLATT and LU, 1936) was one of infantile beriberi. In this case most marked biochemical changes were noted. The statement was made in this paper that infantile beriberi was probably one of the purest forms of vitamin B_1 deficiency seen in human subjects.

Two other points I would like to comment on.

Firstly, with regard to the so-called atrophic form. I think there is good evidence for the belief that in the development of this condition there are two important factors. One is that the deficiency has persisted for a long time. The other is that there have not been present those intensifying factors which I pointed out were operative in the development of the acute symptoms.

Secondly, in connection with water retention, there is a point of some interest. With the disturbance of metabolism, there might well be changes in the water-binding powers of the cells resulting in an increase of intracellular fluid. The first appearance in the muscle tissue is observed as a hardening of

the muscles not unlike that seen in pseudo-hypertrophic muscular dystrophy. WENCKEBACH has described how, when an incision is made over the calf muscles, the muscles obtrude themselves through the opening. There is, however, no evidence of oozing of fluid. On the other hand, in the grossly wet form of beriberi, when there is some degree of heart failure, if not also of kidney failure, there is no question that considerable amounts of fluid may be obtained by acupuncture.

Professor Hawes: With regard to beriberi in infants, we are finding it easier to prevent attacks by giving the injection to the mother, probably before childbirth or when feeding the baby. The mothers frequently have no signs of beriberi, yet the infant will develop an acute attack and die.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

COMMUNICATIONS.

A STUDY OF THE REACTION RATE OF THE SERUM-FORMALIN REACTION IN *TRYPANOSOMA GAMBIENSE* SLEEPING SICKNESS.

BY

C. W. HOPE-GILL, B.M., B.Ch.,
Lately attached Tsetse Investigation, Nigeria.

During recent years attention has been turned to the reaction of GATÉ—better known as the serum-formalin reaction—as an aid in the diagnosis of human trypanosomiasis. Much work has already been done with this reaction in the diagnosis of kala-azar with encouraging results. Trypanosomiasis shares with kala-azar the distinction of producing an exceptional amount of globulin and euglobulin in the blood serum; and, as would therefore be expected, a marked and often rapid gel is formed on the addition of formalin to the sera of patients suffering from these diseases.

It has been shown by MORRISON (1924) and JOHNSON (1925) that from a qualitative point of view the test fails in the diagnosis of trypanosomiasis on account of other pathological conditions producing a positive reaction after a few hours. There is evidence that these globulins are also increased to some extent in other diseases and it therefore is not surprising that under these conditions a gel is also formed by this reaction. The reaction has accordingly—on this account—fallen into some discredit, but a careful study of the test reveals the fact that in trypanosomiasis the gel usually takes a much shorter time to form than in these other pathological conditions.

DYE (1926) working with the *Trypanosoma rhodesiense* type of the disease showed that some value lay in the test when regarded as a time reaction. LEDENTU and VAUCEL (1927), working with the *T. gambiense* type at the Pasteur Institute, Brazzaville, have more recently emphasized the value of the test when the reaction rate is taken into consideration. A series of tests was undertaken, with the reaction rate as the principal factor of importance. The observations, A—G, were performed on a total of 431 subjects. The majority of these were natives from Kano, Bauchi, and Plateau provinces, Nigeria, the remainder being out-patients of the Venereal Diseases Department of St. Thomas's Hospital, London. I was enabled to examine the latter through the courtesy of Dr. T. E. OSMOND, Pathologist to the Department.

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DIAGRAM I.

TIMED RESULTS OF SERUM-FORMALIN REACTION ON

SERIES 1. ——·——·—— 115 POSITIVE S S CASES (T. GAMBIENSE +)

SERIES 2. —·—·—·— 41 APPARENTLY HEALTHY NATIVES IN FLY-FREE COUNTRY.

SERIES 3 70 OUT PATIENTS IN ENDEMIC S S COUNTRY OTHER THAN DETECTED CASES OF SLEEPING SICKNESS

A.—A Suggested Standard Technique for the Performance of the Serum-Formalin Reaction.

Preliminary series of tests, not here recorded, were made in order to ascertain the optimum conditions for the reaction as applied to human trypanosomiasis. It was found that in 1 c.c. of serum 2 minims of commercial formalin gave a more rapid gel than the usual 1 minim, while the reaction was not accelerated by increasing the number of minims above 2.

The *technique* therefore was as follows: Into a small test tube, ½ an inch in diameter (internal) by 2 inches in length, was pipetted 1 c.c. of serum or citrated plasma, to which was added 2 minims of 40 per cent. (commercial) formalin. The test tube was then thoroughly shaken on account of the formalin having a tendency to settle at the bottom of the tube, so resulting in a delayed gel. It was important that for these timed reactions there should be a definite and uniform end-point. This was determined by gently inverting the tube without shaking. Failure of the contents to run down the side of the tube was regarded as a positive result.

Uniformity in the diameter of the test tube is therefore essential.

B.—Estimation of the Value of the Serum-Formalin Reaction in the Diagnosis of Human Trypanosomiasis.

For this purpose tests were performed on the following groups: *Series* 1—Positive sleeping sickness cases (*T. gambiense* demonstrated); *Series* 2—Apparently healthy natives in fly-free country; *Series* 3—Out-patients other than detected cases of trypanosomiasis.

Series 1.—*Positive Sleeping Sickness Cases.*

This series shows the results of timed formol-gel reactions on 115 cases of sleeping sickness. These are termed positive sleeping sickness cases as *T. gambiense* had been found in the gland juice. They had all attended voluntarily at bush clinics, and were therefore well-established cases of the disease, early cases being very seldom seen.

Diagram 1, Series 1, shows that a positive gel was given within 5 minutes by 40 per cent.; 10 minutes by 59 per cent.; 15 minutes by 72 per cent.; 30 minutes by 82 per cent.; and 60 minutes by 93 per cent. of positive sleeping sickness cases, while 7 per cent. failed to gel within 60 minutes.

Series 2.—*Apparently Healthy Natives.*

Human trypanosomiasis is so ubiquitous in Kano, Bauchi, and Plateau provinces that it was not easy to obtain a series guaranteed free from latent or active sleeping sickness. These conditions were fulfilled by the village of Richa, on the high ground of the plateau and at some distance from fly country. The pagan population were local in their habits, and examination showed them to be free from trypanosomiasis.

DIAGRAM I.

TIMED RESULTS OF SERUM-FORMALIN REACTION ON

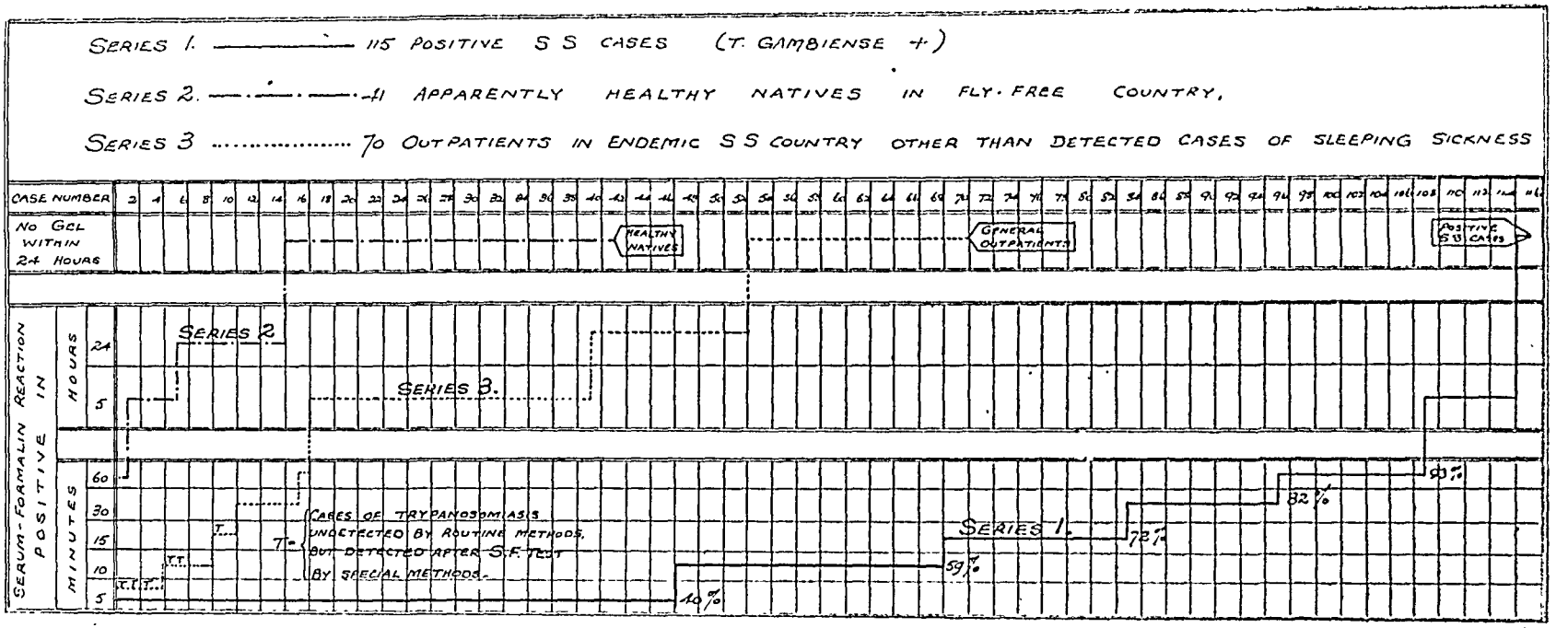


Diagram 1, Series 2, shows that out of forty-one people drawn from the general population of Richa one gave a positive gel within 60 minutes. Nearly 98 per cent. therefore failed to give a positive reaction within 60 minutes.

Series 3.—Out-Patients other than Detected Cases of Trypanosomiasis.

Cases in this series were tested and examined at Gadau, Nigeria, headquarters of the Tsetse Investigation. The patients at Gadau clinic were drawn from the surrounding country where sleeping sickness is endemic. Diagram 1, Series 3, shows that out of a total of seventy out-patients other than cases of detected trypanosomiasis, 16 gave a positive gel within varying periods up to 60 minutes. The latter were not suspected of trypanosomiasis before the serum-formalin reaction was performed. Of the sixteen cases that were positive within 60 minutes one case of leprosy went away before further investigation could be made. The remaining fifteen cases were examined further in order to discover whether any were suffering from undetected trypanosomiasis, as follows :—

1. *Case* 10.—Male aged 40. Seven years' history of debility, serum-formalin test positive in 15 minutes. No glands palpable. Blood, negative. Cerebrospinal fluid : 147 white cells per cmm. : Pandy's phenol globulin and acetic-anhydride tests positive. Kahn test negative. *Diagnosis* : *Trypanosomiasis.*

2. *Case* 18.—Male aged 35. Four years' history swollen abdomen (ascites). S.F. test positive in 30 minutes. No glands palpable. Blood and C.S.F. examination negative. 10 c.c. blood inoculated into peritoneal cavity of monkey (*Macacus rhesus*) : monkey's blood examined for 6 weeks with negative result.

3. *Case* 19.—Female aged 40. Three years' history swollen abdomen (ascites). S.F. test positive in 30 minutes. Examination and monkey inoculation as in Case 18 negative. Test for adhesion phenomenon negative.

4. *Case* 22.—Male aged 35. Six years' history of debility. S.F. test positive in 30 minutes. Kahn test negative. Examination and monkey inoculation as in Case 18 negative. Test for adhesion phenomenon negative.

5. *Case* 40.—Male aged 30. Leg ulcer of 3 weeks' duration. S.F. test positive in 5 minutes. Kahn test positive. Examination of C.S.F. : white cell count of 16 per cmm. : Pandy's and acetic-anhydride tests for globulin positive. Was later proved to have been a recently discharged sleeping sickness case. *Diagnosis* : *Trypanosomiasis.*

6. *Case* 49.—Male aged 40. Treated for secondary syphilis. S.F. test positive in 5 minutes. Kahn test positive. *T. gambiense* was afterwards found in gland juice and blood. C.S.F. examination : white cell count 15 per cmm. : tests for increased globulin positive. *Diagnosis* : *Trypanosomiasis.*

7. *Case* 55.—Male aged 37. Two months' history of debility. S.F. test positive in 10 minutes. Examination of glands, blood, and C.S.F. negative. Test for adhesion phenomenon positive. *Diagnosis* : *Trypanosomiasis.*

8. *Case* 57.—Male aged 35. Bright's disease with much free fluid. S.F. test positive in 5 minutes. Examination of blood and C.S.F. negative. Glands not palpable. Test for adhesion phenomenon negative.

9. *Case* 66.—Male aged 35. Three years' history of debility. S.F. test positive in 20 minutes. Kahn test positive. Adhesion phenomenon test negative.

10. *Case* 65.—Male aged 27. Secondary syphilis. S.F. test positive in 30 minutes. Kahn test positive. Test for adhesion phenomenon negative.

11. *Case* 73.—Male aged 31. Three years' history of gonorrhoea. S.F. test positive in 20 minutes. Kahn test negative. Test for adhesion phenomenon negative.

12. *Case* 85.—Male aged 25. One year's history of pain and swelling of right hip joint. S.F. test positive in 60 minutes. Kahn test negative. Test for adhesion phenomenon negative.

13. *Case* 88.—Male aged 31. Amoebic dysentery. S.F. test positive in 10 minutes. Kahn test negative. Test for adhesion phenomenon negative.

14. *Case* 107.—Male aged 30. Seven years' debility. S.F. test positive in 5 minutes. Kahn test negative. Examination of C.S.F.: white cell count 228 per cmm.: globulin tests positive. *Diagnosis*: *Trypanosomiasis.*

15. *Case* 125.—Male aged 40. Five years' history of debility. S.F. test positive in 10 minutes. C.S.F.: *T. gambiense* positive: white cell count 148 per cmm.: globulin positive. *Diagnosis*: *Trypanosomiasis.*

Six cases, Cases 10, 40, 49, 55, 107 and 125, are thus classed as undetected cases of trypanosomiasis. The remaining nine are taken to be free from the disease on the findings of the adhesion phenomenon and inoculation of blood into monkeys. Nine out of the series of sixty-nine are therefore classified as false positives (13 per cent.). It should be stated that no provocative doses of tryparsamide were administered before the test for the adhesion phenomenon. With regard to these results with the adhesion phenomenon DUKE and WALLACE (1930) reached the conclusion that a single, or even a number of, negative observations do not exclude trypanosomiasis: a positive reaction on the other hand indicates recent or actual infection with a trypanosome of the same group. I am indebted to Dr. H. M. O. LESTER for performing these tests for the adhesion phenomenon.

The following data are now available:—

(1) 93 per cent. of proved cases of human trypanosomiasis have given a positive serum-formalin reaction within 60 minutes.

(2) Less than 1 per cent. of proved cases of trypanosomiasis have failed to give a positive reaction within 24 hours.

(3) Nearly 98 per cent. of apparently healthy natives free from trypanosomiasis have failed to give a positive reaction within 60 minutes.

(4) 13 per cent. of patients suffering from pathological conditions other than trypanosomiasis have given a positive reaction within 60 minutes.

C.—THE INFLUENCE, IF ANY, OF CERTAIN THERAPEUTIC DRUGS ON THE SERUM-FORMALIN REACTION WHEN ADDED TO THE SERUM *in vitro*.

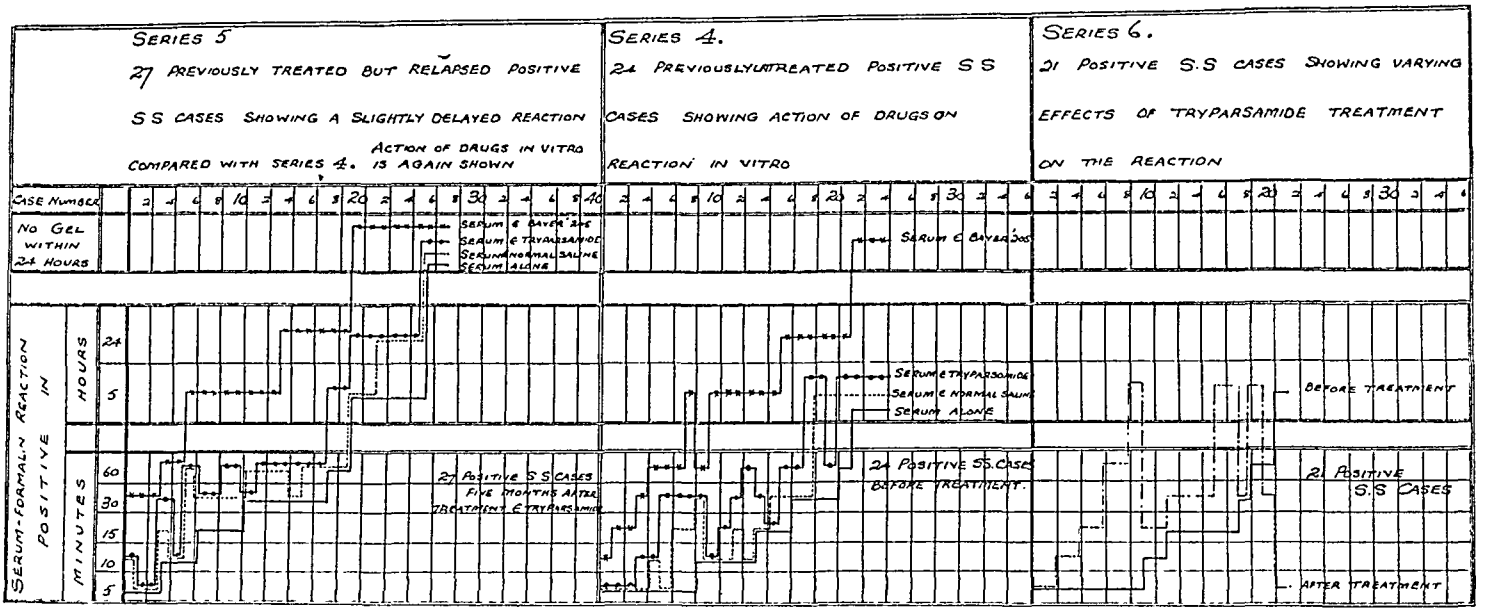
The drugs tested were Bayer 205, tryparsamide, and neosalvarsan. Normal saline was used as a control.

Series 4.—Positive Sleeping Sickness Cases.

The serum was obtained from 24 previously untreated positive sleeping sickness cases. Five test tubes were necessary for each serum tested. Into each test tube was pipetted 1 c.c. of the serum to be tested. Into each of four test tubes respectively was dropped 2 minims of freshly prepared 10 per cent. Bayer 205, 10 per cent. tryparsamide, 10 per cent. neosalvarsan, and normal saline. The fifth test tube contained 1 c.c. of serum only. After shaking, to

Diagram II.

TIMED RESULTS OF SERUM-FORMALIN REACTION ON



each of the five test tubes was added 2 minims of 40 per cent. formalin as in the normal performance of the test. The resulting reactions were timed and are recorded in Diagram II, Series 4.

The results with Bayer 205 are comparable with those obtained by DYE (1926), the reaction being considerably delayed in each case. With tryparsamide the reaction was delayed slightly more than was the case with the normal saline control. The results with neosalvarsan differed very slightly from those with normal saline, and are not recorded.

It is shown, therefore, that Bayer 205 *in vitro* exercises a considerable retarding effect upon the reaction. It remains to discover whether this retarding action is exercised when other pathological sera are used, or whether the action is selective for the serum of human trypanosomiasis. The retarding action of tryparsamide is too slight to be of possible value, while that of neosalvarsan is negligible.

D.—The Influence of Previous Treatment with Tryparsamide upon the Reaction when using Serum alone, and when the above Drugs are added *in vitro*.

Series 5.—*Previously Treated Positive Sleeping Sickness Cases.*

These had undergone an insufficient course of treatment with tryparsamide about 5 months previously, and had relapsed. Diagram II, Series 5, shows that the reaction rate under these circumstances is definitely retarded. The action *in vitro* of Bayer 205 and tryparsamide was also tested as in Series 4, and shows a similar retarding effect in relation to the results obtained when using serum alone.

Accordingly, in performing the serum-formalin reaction on cases of trypanosomiasis the question of previous treatment with tryparsamide should be considered. In cases which have undergone previous treatment, the possible retarding effect of this should be borne in mind.

E.—The Value of the Test as an Indication of the Progress of a Case of Trypanosomiasis under Treatment.

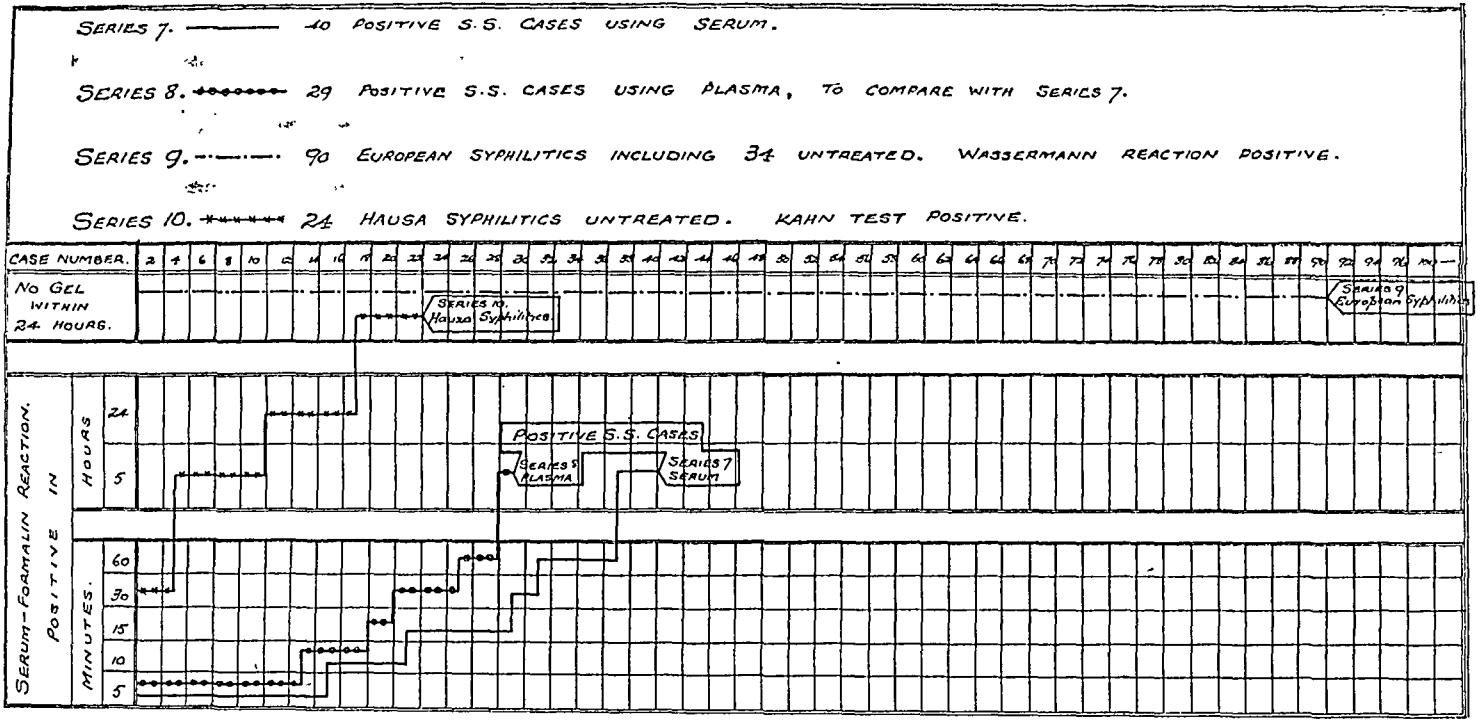
Series 6.—*Positive Sleeping Sickness Cases.*

Each case of this series was under daily observation, and the clinical progress was carefully noted and recorded. The serum-formalin test was performed before treatment, and again at the end of a course of tryparsamide based on a dosage of 20 grammes per adult. Referring to Diagram II, Series 6, the continuous line indicates the timed results of the test before treatment, and the dotted line the results after treatment. In the majority of cases the effect of treatment was to delay the reaction, but results were variable, and often did not coincide with the records of clinical progress.

It is concluded that the test affords no reliable indication of the progress of a case of trypanosomiasis under treatment.

DIAGRAM III.

TIMED RESULTS OF SERUM-FORMALIN REACTION ON



F.—A Comparison of the Use of Serum and Citrated Plasma Respectively in the Performance of the Test.

Series 7.—Positive Sleeping Sickness Cases.

Diagram III, Series 7, shows the timed results of the serum-formalin reaction when serum was used.

Series 8.—Positive Sleeping Sickness Cases.

Diagram III, Series 8, shows the results similarly timed when citrated plasma was used.

Similar results are obtained in each series so that serum and citrated plasma are equally suitable for the performance of the test.

G.—Estimation of the Influence of Syphilis upon the Serum-Formalin Reaction.

Series 9.—European Syphilitics.

These were out-patients of both sexes from the Venereal Diseases Department of St. Thomas's Hospital. In each case the Wassermann reaction was positive. All stages of syphilis are included as shown in the table below. Thirty-four of these had had no previous anti-syphilitic treatment, while the remainder were in various stages of treatment. In every case the serum showed no gel within a period of 24 hours.

Table.

Serum-Formalin Reaction on Sera of European Syphilitics.
Wassermann Reaction Positive.

Stage of Disease.	Under Treatment.	Previously Untreated.
Primary	3	11
Secondary	6	14
Tertiary	24	4
Latent	13	3
Congenital	4	2
Tabes dorsalis	6	0
Totals	56	34
Total number tested	90	
Results of test	No gel within 24 hours in every case.	

Series 10.—*Hausa Syphilitics.*

These were Hausas of both sexes. In every case the Kahn test was strongly positive. No previous treatment had been given. In performing the Kahn test two tubes were used instead of three, the strongest serum-suspension mixture being eliminated. The results of the timed serum-formalin reactions in Series 9 and 10 are demonstrated in Diagram III.

It is evident, therefore, that in performing the serum-formalin test for the diagnosis of trypanosomiasis the influence of syphilis as a disturbing factor is negligible.

Conclusions and Comments.

1. The serum-formalin reaction, when positive within 60 minutes under the conditions described, is strong evidence in favour of the presence of trypanosomiasis; when negative within this time limit it is strong evidence against the presence of this disease; and when negative after 24 hours is practically diagnostic of the absence of the disease.

2. A serum-formalin reaction positive for trypanosomiasis is, therefore, one which within an hour gives a positive gel according to the technique described in this paper. This would apply to a country such as tropical Africa where *T. gambiense* sleeping sickness but not kala-azar, which also gives a positive reaction, is endemic.

3. The presence of Bayer 205 in the serum *in vitro* considerably retards the end-point of the reaction.

4. A retarding effect is also caused by previous treatment with tryparsamide.

5. The serum-formalin reaction gives no reliable information as to the progress of a case of human trypanosomiasis under treatment.

6. The reaction takes place equally well whether serum or citrated plasma is employed.

7. Interference by the presence of syphilis is negligible.

8. These tests were carried out on the sera of patients who presented themselves voluntarily for treatment in a strongly endemic area, and the majority were therefore comparatively advanced cases of trypanosomiasis. It remains to be shown what results would be obtained in cases diagnosed in the course of a mass examination where many cases complain of no symptoms and are often only mildly affected by the disease.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

MORPHOLOGICAL AND TAXONOMIC STUDIES ON MAMMALIAN TRYPANOSOMES.

IV. BIOMETRICAL STUDY OF THE RELATIONSHIP BETWEEN *Trypanosoma uniforme* AND *T. vivax*.

BY

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AND

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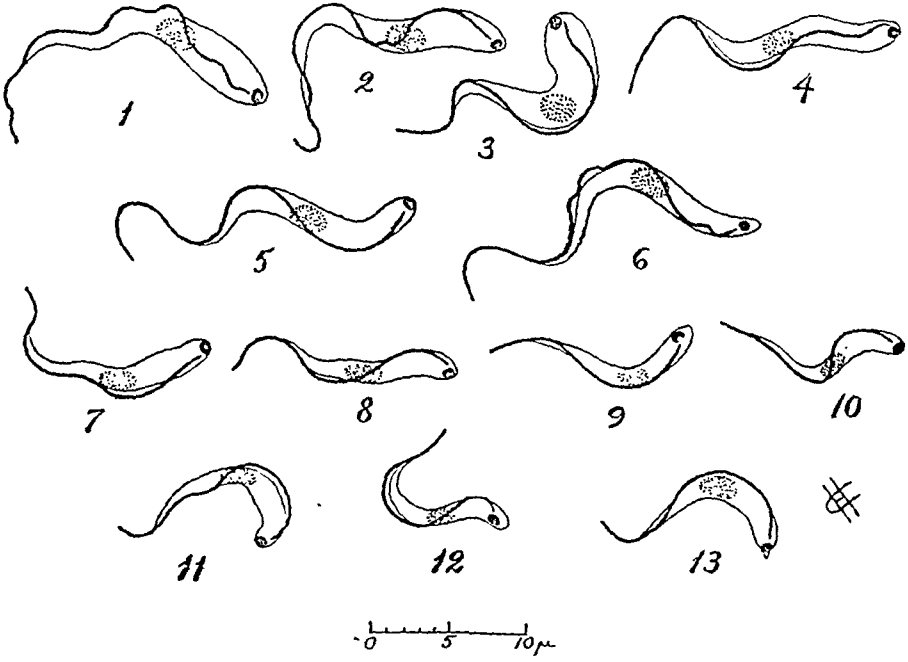
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I.—INTRODUCTION.

Amongst the pathogenic mammalian trypanosomes the *vivax* group is more clearly defined than the other groups, in which there occurs a certain degree of morphological overlapping. Thus, the trypanosomes of the monomorphic *evansi* group are similar to the "long" forms of the polymorphic *brucei* group which can be differentiated from the former by the presence of the "short stumpy" forms. However, old laboratory strains of the polymorphic trypanosomes—in which the "short" forms have disappeared—become morphologically very similar to, if not indistinguishable from, trypano-

somes of the *evansi* group. As regards the *congolense* group, the short forms of *Trypanosoma congolense* are not unlike the " stumpy " trypanosomes in the *brucei* group, while some forms of *T. simiae* resemble the " intermediate " forms in the last named group.

The *vivax* group is represented by monomorphic trypanosomes with a peculiar combination of characters not found in the groups mentioned above. The body is somewhat club-shaped, being typically swollen and rounded at the posterior end and tapering towards the anterior (flagellar) end. The undulating membrane is usually very feebly developed (except in *T. caprae*, as described by BRUCE *et al.*: *v. infra*) and there is a distinct free flagellum. The kinetoplast* is typically terminal (Text-Fig. A, 1, 3, 5, 7, 10, 11), but



TEXT-FIG. A: 1 to 6—*T. vivax* (1, 2—caprine strain, Nigeria; 3, 4—bovine, Uganda; 5, 6—bovine, Mauritius). 7 to 13—*T. uniforme* (7, 8, 9—bovine strain, Belgian Congo; 10, 11—situtunga, Uganda; 12, 13—bovine, Uganda). (All figures were drawn with the aid of a camera lucida at × 2,000.)

may be subterminal, in which case it is usually also marginal or lateral (Text-Fig. A, 2, 8, 9, 12). The most remarkable feature of the kinetoplast is its size, which is considerably greater than that of the kinetoplast in any of the groups referred to above (*evansi*, *congolense*, *brucei*). In this respect it is comparable to the dimensions of this structure in members of the *lewisi* group.

* We have used the term " kinetoplast " in its original sense (cf. ALEXEIEFF: *C.R. Soc. Biol.* 80. 1917. 512), to denote the kinetonucleus alone (without the blepharoplast).

Though the large size of the kinetoplast in the *vivax* group is well known, its diagnostic value does not seem to have been sufficiently appreciated.

The biological properties of all the members of the *vivax* group are similar: they are infective to various wild and domestic ungulates, but not to laboratory rodents (except the rabbit, which is to some extent susceptible to *T. vivax*), and they are transmitted by tsetse-flies in which their development takes place in the proboscis exclusively.

Of the three species comprising the *vivax* group (*T. vivax*, *T. caprae*, *T. uniforme*) *T. vivax* is the best known and is commonly encountered in different parts of tropical Africa, as well as in Mauritius and possibly in South America (*T. viennei*). Very little is known about *T. caprae*, which has so far been recorded only from the former German East Africa and from Nyasaland, and which—as far as we are aware—has not been reported again since 1914. This trypanosome has the same length and general appearance as *T. vivax*. It should be noted, however, that there is a discrepancy between the description of *T. caprae* as given by KLEINE (1910) and KLEINE and TAUTE (1911), on the one hand, and that by BRUCE and his collaborators (1913) on the other hand. In the account and figures of the German authors the trypanosome is indistinguishable from *T. vivax*, and—like *T. bovis* (= *T. vivax*) recorded in the same publications—it evidently owes its name merely to the host in which it was found. However, the trypanosome described by the British authors differs both from *T. vivax* and from KLEINE'S *T. caprae* in the possession of a well-developed undulating membrane and in the heavier (broader) build of its body.

It would thus appear that KLEINE'S trypanosome is identical with *T. vivax* and no more available under the Rules of Zoological Nomenclature. In that case, the name *T. caprae* Kleine, 1910, being actually a synonym of *T. vivax*, a new name should be given to the trypanosome described by BRUCE *et al.* under the name "*T. caprae.*" It is curious that these facts which are immediately apparent on comparison of the original figures illustrating *T. vivax* and *T. caprae* (*sensu* KLEINE and BRUCE respectively) have escaped the attention of all previous authors. The problem of *T. caprae* can, however, only be settled by re-examination of the trypanosomes in question, and need not concern us any further in this paper.

The remaining species of this group, *T. uniforme*, with which we are mainly concerned in this work, will be dealt with in greater detail in the next section.

II.—RECORDS OF *T. uniforme*.

T. uniforme was first described and named by BRUCE *et al.* (1911) who discovered it in several bovines in Uganda. *T. uniforme* was subsequently recorded from antelopes (bushbuck, situtunga, waterbuck) and buffalo in Uganda (FRASER and DUKE, 1912a, 1912b*; DUKE, 1912, 1913a, 1913b, 1916).

*According to a personal communication received from Dr. H. L. DUKE, he and FRASER recognized the trypanosome studied by them as a new species independently of BRUCE and his co-workers, and later identified it with the newly-created *T. uniforme*.

This trypanosome was also reported from bovines in Zululand (CURSON, 1928) and in the Belgian Congo (SCHWETZ and STORCK, 1930; SCHWETZ, 1931). In Uganda *T. uniforme* was again recovered from situtunga by one of us (HOARE, 1932*) and from bovines by Dr. G. N. HALL.† Finally there is some evidence of the occurrence of *T. uniforme* in the domestic pig (a mixed infection with *T. simiae*: cf. HORNBY, 1923; HOARE, 1936b).

The naturally infected hosts recorded up to the present and the geographical distribution of *T. uniforme*, checked from the original sources, are summarized in the following list.

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The records of *T. uniforme* are thus seen to be relatively scanty and its distribution restricted, for, with the exception of the isolated findings in Zululand and Tanganyika, it has hitherto been found only within a limited area extending from the northern shores and islands of Victoria Nyanza into western Uganda and, over the border, into eastern Belgian Congo.

The apparent rareness of this trypanosome, on the one hand, and its similarity to *T. vivax*, on the other, have led to some scepticism regarding the independent status of *T. uniforme*. This attitude found expression at two conferences on trypanosomiasis recently held in Entebbe, Uganda.

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‡Like the bushbuck, the situtunga was formerly included in the genus *Tragelaphus*, from which it was subsequently removed to the genus *Limnotragus*. However, in view of the successful hybridization between these two antelopes (cf. H. L. DUKE, 1934, *Uganda J.*, 2, 159) it would appear that they should both be retained in the same genus, *Tragelaphus*.

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The question of *T. uniforme* was again raised at the " Conference on the Co-ordination of Tsetse and Trypanosomiasis (animal and human) Research in East Africa " in 1936. HORNBY (*loc. cit.*, p. 27) proposed that " those who favour the retention of this name [*T. uniforme*] as valid should at least furnish stained specimens which can be critically compared with similar specimens of *T. vivax*," adding : " It is significant that, in spite of increased work on trypanosomiasis throughout Africa, not a single article dealing with *T. uniforme* appears to have been published in recent years." METTAM (*loc. cit.*, p. 18) reiterated his opinion that *T. uniforme* was probably a synonym of *T. vivax*, and the Conference again agreed that this question stands in need of reinvestigation.

Unfortunately, METTAM provided no evidence in support of his contention, either in the reports of the Conferences or in any other publication. As regards HORNBY'S second remark, it is obvious that he had overlooked the recent observations on *T. uniforme* in the Belgian Congo (SCHWETZ and STORCK, 1930 ; SCHWETZ, 1931) and the record from Zululand (CURSON, 1928). It is true, however, that this species had not been reported between 1916 and 1928.

It is our firm belief that the sporadic nature of the records of *T. uniforme* is due not so much to its rareness or to its focal distribution, as to failure on the part of observers to recognize and differentiate this species. As soon as its presence was established in Uganda it was found to be the most common trypanosome on the northern shore and islands of Lake Victoria (DUKE, 1912, 1913a). Its continued presence there in 1927 and 1928 was confirmed by the findings mentioned above (HALL, HOARE : cf. p. 520). According to SCHWETZ (1931) this species ranks second in importance as a parasite of cattle in the Stanleyville district of the Belgian Congo.*

Moreover, both in Uganda and in the Congo *T. uniforme* was found to be co-existent with *T. vivax*, the two species occurring not only in the same localities, but in mixed infections of the same host. In the case of such double infections it is evident that the differentiation of the two trypanosomes presents no difficulty.

While some observers (HORNBY, METTAM) appear to doubt the very existence of *T. uniforme* as a distinct parasite, others—though recognizing its peculiarities—are doubtful about its specific independence. Thus, according

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2. *Trypanosoma uniforme.*

Strain H : Situtunga, from Uganda (*ppt.* C. A. HOARE, 1928).
Strain I : Bovine, from Buvuma Island, Uganda (*ppt.* Dr. G. N. HALL, 1927).
Strain J : Bovine, from Buvuma Island, Uganda (*ppt.* Dr. G. N. HALL, 1927).
Strain K : Bovine (No. 87), from Belgian Congo (*ppt.* Dr. J. SCHWETZ, 1928).
Strain L : Bovine (No. 18), from Belgian Congo (*ppt.* Dr. J. SCHWETZ, 1928).

Since the only appreciable difference between *T. vivax* and *T. uniforme* is the length of their body, this was the only character taken into consideration in comparing the two species. In each of twelve strains the length of 100 non-dividing individuals was measured*, taking the trypanosomes in the order in which they appeared in view. The measurement of each was made by drawing (with the aid of a camera lucida at $\times 2{,}500$) a line running through the middle of the body of the trypanosome, from the posterior end to the tip of the flagellum, and measuring this line with a divider set at 1/20 inch ($=$ ca. $0{\cdot}5\mu \times 2{,}500$†).

One hundred measurements were originally made on a few strains as a preliminary, but, as will be shown later, it proved possible to differentiate most of the strains with these figures, so that larger numbers were considered unnecessary.

To compare the strains and species with each other the Arithmetic Mean or Average (M) of each series of measurements, and the Standard Errors of the Differences between the means (σ_D) were calculated. The latter characteristics are obtained as follows: From the Variance (V) of each series, which is the mean of the squares of the deviations of the values from the arithmetic mean, the Variance of the Mean (V_M) is obtained by dividing the variance (V) by the number of observations in the series. The variances of the two means to be compared are added together and the square root of their sum is the Standard Error of the Difference between the means, i.e., $\sigma_D = \sqrt{V_{M_1} + V_{M_2}}$. The standard errors of the differences are, of course, calculated separately for each pair of means.

When the difference between two means exceeds three times the standard error the means are considered to differ significantly and the two series of measurements cannot have been drawn from the same original "population." Or, in other words, as regards the present case, that there is a real difference between the two strains. The results obtained by applying the test are described later.

*In the case of the Tanganyika strains of *T. vivax* (M and N), the preparations of which were received when this paper was ready for the press, only ten individuals of each have been measured, according to the method described on p. 531.

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to LAVERAN and MESNIL (1912: p. 552): "Le *Tr. uniforme* est très voisin du *Tr. Cazalboui* [= *T. vivax*]; peut-être les deux trypanosomes appartiennent-ils à la même espèce"; WENYON (1926: p. 565) says: "It is open to question whether they [*T. vivax*, *T. caprae* and *T. uniforme*] represent distinct species or should be regarded as merely varieties or races of *T. vivax*"; SCHWETZ (1931) characterizes *T. uniforme* as "un petit *T. vivax*," and raises the same question: "S'agit-il de deux espèces différents ou *T. vivax* et *T. uniforme* ne sont-ils que deux variétés de la même espèce . . . ?"

From the foregoing account it is seen that the systematic position of *T. uniforme* is still a subject of controversy and stands in need of further investigation on a larger scale and by more exact methods than has hitherto been attempted. In the past the systematics of trypanosomes has suffered both from the separation and from the union of species on inadequate grounds (examples of the former case are provided by the *brucei* and *evansi* groups, and of the latter by the *congolense* group). The same fate threatens the members of the *vivax* group, unless their position can be defined one way or another, according to the recognized principles of taxonomy.

Having secured a representative collection of blood-films of both *T. uniforme* and *T. vivax* we have undertaken a comparative morphological study of these two trypanosomes by statistical methods, in the hope of elucidating their true relationship.

III.—MATERIAL AND METHODS.

The material at our disposal consisted of blood-films (stained by one of the Romanowsky methods) taken at various periods, in different localities, from diverse natural hosts (except in the case of Strain G: *v. infra*), and representing altogether fourteen strains, as shown below.*

1. *Trypanosoma vivax.*

Strain A: Bovine (Ox E), from Mauritius (*praeparavit* Dr. A. R. D. ADAMS, 1935).
Strain B: Bovine (Ox A), from Mauritius (*ppt.* Dr. A. R. D. Adams, 1934).
Strain C: Bovine, from Uganda (*ppt.* Dr. J. CARMICHAEL, 1934).
Strain D: Bovine (No. 9415), from Northern Nigeria (*ppt.* Dr. G. N. HALL, 1936).
Strain E: Bovine (No. 9352), from N. Nigeria (*ppt.* Dr. G. N. HALL, 1936).
Strain F: Caprine, from N. Nigeria (*ppt.* Dr. G. N. HALL, date ?).
Strain G: Rabbit (1st passage from bovine), from N. Nigeria (*ppt.* Dr. G. N. HALL, 1936).
Strain M: Bovine (No. 4730), from Tanganyika (*ppt.* Mr. H. E. HORNBY, 1937).
Strain N: Bovine (No. 3626), from Tanganyika (*ppt.* Mr. H. E. HORNBY, 1937).

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†Actually 1/20 inch = 0·49125μ ×2,500.

KARL PEARSON (1914), who made an extensive analysis of BRUCE's measurements of the *brucei* group of trypanosomes, concluded that a more adequate criterion of identity of strains is furnished by the χ^2 test which compares the distribution of the individuals throughout the whole range of measurements. This method of comparison was therefore used also.

When comparing two strains by this test the measurements are first grouped into classes. The classes chosen depend on the number in the series and the range of the measurements, but in the present instance intervals of 1μ were adopted. Thus, for example, all lengths between 15μ and 16μ were grouped together in one class, and so on. Each class is then treated separately. The difference between the numbers of the two strains in the class is squared and then divided by the total number in the class. These quotients are added to give χ^2. The probability that such a value of χ^2 could arise by chance between two samples drawn from the same population is obtained from appropriate tables. If the probability is less than 0·01, a real difference is assumed to exist between the two strains. The results of this test also are referred to below.

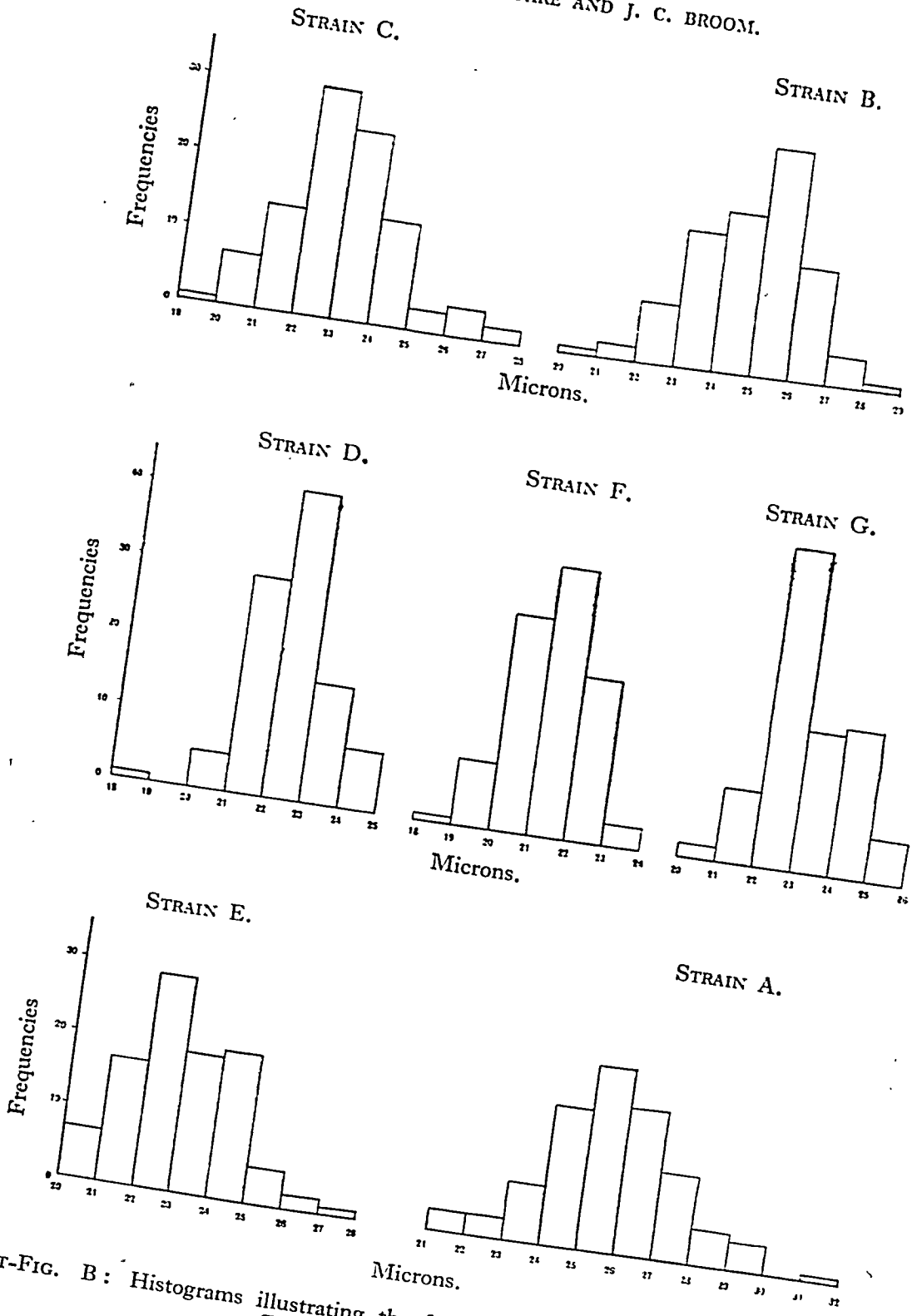
IV.—MEASUREMENTS OF *T. vivax*.

The distribution in respect of length of 100 individuals of each strain of *T. vivax* (except M and N), grouped in classes of 1μ, is given in Table I and illustrated in Text-Fig. B.

TABLE I.

T. vivax: FREQUENCY DISTRIBUTION OF 7 STRAINS IN RESPECT TO LENGTH.

Strains.	Length in Microns.														Total No. Measured.	Mean Length.
	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
A	—	—	—	3	3	8	19	25	20	12	5	4	0	1	100	25·4
B	—	—	1	2	8	18	21	30	15	4	1	—	—	—	100	24·5
C	—	1	7	14	30	25	14	3	4	2	—	—	—	—	100	22·7
D	1	0	5	29	41	16	8	—	—	—	—	—	—	—	100	22·1
E	—	—	7	17	29	19	20	5	2	1	—	—	—	—	100	22·7
F	1	9	29	36	22	3	—	—	—	—	—	—	—	—	100	21·0
G	—	—	2	10	43	19	20	6	—	—	—	—	—	—	100	22·8
Total	2	10	51	111	176	108		69	41	19	6	4	0	1	700	



TEXT-FIG. B: Histograms illustrating the frequency distribution of seven strains of *T. vivax* in respect of length.

In the histograms the area of each rectangle is, of course, proportional to the number of measurements in the corresponding class. From the table it is seen that the mean lengths of the strains vary from 21·0μ to 25·4μ and the complete range of length is from 18μ to 31μ, though over 90 per cent. of the measurements lie within the limits 20μ to 26μ.

The blood-films of the Tanganyika strains of *T. vivax* (M and N) were received too late for inclusion in the tables and text-figures. The lengths of ten individuals of each strain ranged from 25·5μ to 28·0μ in M, and from 21·5μ to 30·0μ in N, the means of these measurements being 26·7μ and 26·0μ respectively, which is in agreement with the corresponding figures for the other strains of *T. vivax* examined by us (see p. 531).

It is of interest to compare the present findings with those of previous observers. BRUCE *et al.* (1910) examined nine strains of *T. vivax* derived from natural infections in cattle. The mean length of twenty individuals varied between 21·4μ and 26·5μ, which is in accord with our means of ten measurements which range from 20·5μ to 27·3μ (see p. 531). BLACKLOCK (1912) inoculated a single strain into a number of goats and measured 1,000 trypanosomes in groups of twenty taken from different animals at various stages of the disease. The means of the groups are not all given, but he states that " in each set dealt with . . . the largest number of trypanosomes constantly lies between 20μ and 23μ."

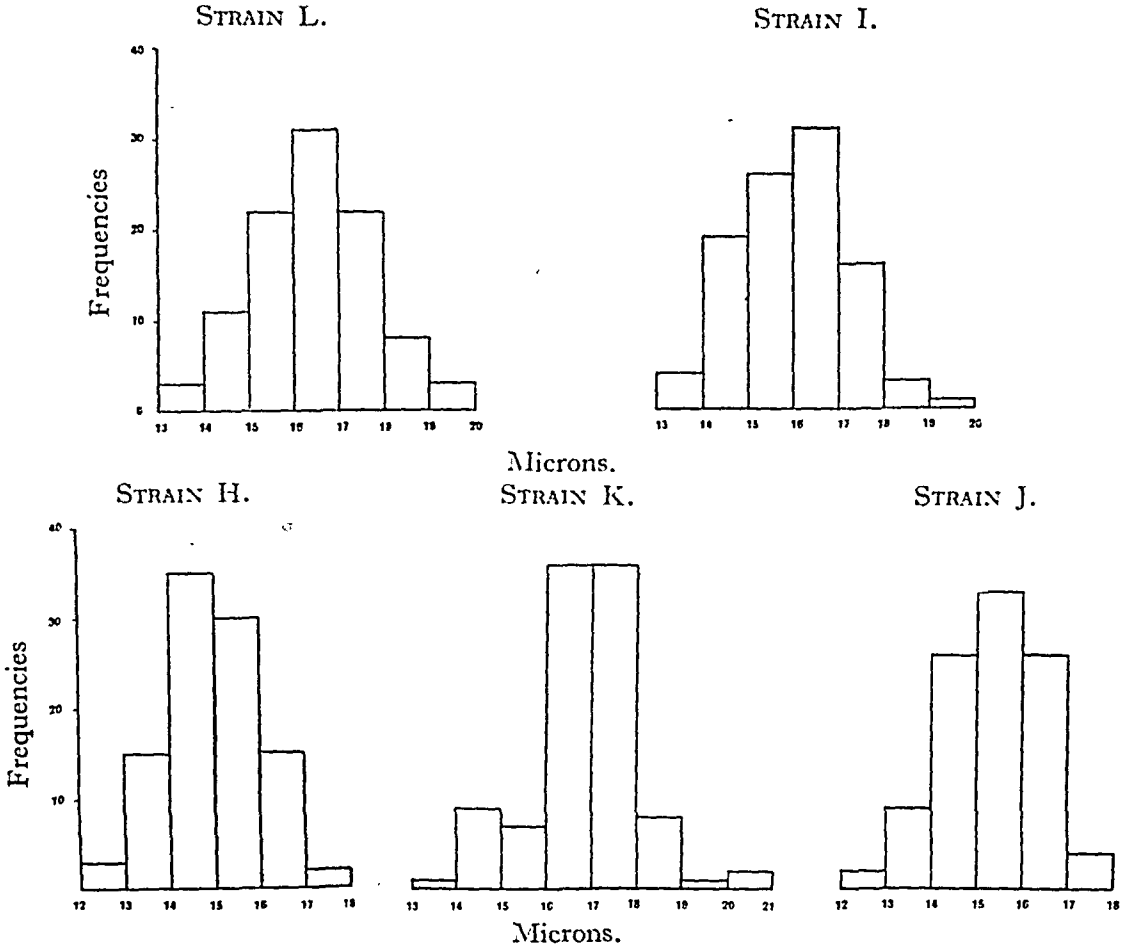
V.—MEASUREMENTS OF *T. uniforme*.

The frequency distributions of the lengths of the strains of *T. uniforme* are shown in Table II and in Text-Fig. C in the same way as in the case of *T. vivax*. Here the means vary from 14·6μ to 16·5μ and the range is 12μ to 20μ, with over 90 per cent. between 13μ and 17μ.

TABLE II.

T. uniforme: FREQUENCY DISTRIBUTION OF 5 STRAINS IN RESPECT TO LENGTH.

Strains.	Length in Microns.									Total No. Measured.	Mean Length.
	12	13	14	15	16	17	18	19	20		
H	3	15	35	30	15	2	—	—	—	100	14·6
I	—	4	19	26	31	16	3	1	—	100	15·7
J	2	9	26	33	26	4	—	—	—	100	15·0
K	—	1	9	7	36	36	8	1	2	100	16·5
L	—	3	11	22	31	22	8	3	—	100	16·1
Total	5	32	100	118	139	80	19	5	2	500	



TEXT-FIG. C : Histograms illustrating the frequency distribution of five strains of *T. uniforme* in respect of length.

As regards earlier observations, BRUCE *et al.* (1911) measured groups of twenty trypanosomes drawn from different animals and representing four original strains. The mean lengths varied between 14·7μ and 17·8μ. FRASER and DUKE (1912b) studied one strain which they inoculated into oxen and goats. Their means of twenty measurements ranged from 14·1μ to 17·2μ. These observations also correspond to our means of ten measurements (see p. 531), which lie between 14·1μ and 17·3μ.

VI.—GENERAL DISCUSSION.

1. STATISTICS.

Before comparing the two species, *T. vivax* and *T. uniforme*, with each other one must determine to what extent the strains of each species differ among themselves. Ideally it would be found that each species could be considered as one population and the strains as samples drawn from it, unaffected by host or previous history and differing from each other only by the errors introduced by random sampling. To decide whether this is a valid assumption

each strain was compared with every other of the same species, using the two tests outlined above.

The probabilities that the differences observed in the means and distributions are due to chance are shown in Tables III to VI.* In Tables III and V the distributions are compared by the χ^2 test. In 14 of the 21 comparisons of *T. vivax* strains and in 4 of the 10 of *T. uniforme* the probability is less than 10^{-6}, i.e., the odds are more than a million to one against such differences being the result of chance. In the other cases the probability is greater, in fact, the difference between strains C and E is so small that in 8 out of 10 samples drawn from the same population similar divergencies would be expected. The results of the tests by comparing the means are shown in Tables IV and VI. The probabilities that the strains are identical are very similar to those obtained by the χ^2 test.

TABLE III.

	A	B	C	D	E	F	G	
A		·005	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	A
B	10^{-4}		$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	B
C	$<10^{-6}$	$<10^{-6}$		10^{-4}	·80	$<10^{-6}$	·11	C
D	$<10^{-6}$	$<10^{-6}$	10^{-6}		·004	$<10^{-6}$	10^{-3}	D
E	$<10^{-6}$	$<10^{-6}$	·86	$<10^{-4}$		$<10^{-6}$	·18	E
F	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-8}$		$<10^{-6}$	F
G	$<10^{-6}$	$<10^{-6}$	·83	$<10^{-5}$	·67	$<10^{-6}$		G
	A	B	C	D	E	F	G	

TABLE IV.

TABLES III and IV.—The probability that the observed differences in the frequency distributions in respect of length (Table III) and the differences of the mean lengths (Table IV) of the strains of *T. vivax* could be the result of chance.

*The tables referring to the differences of the means and the differences in the distributions respectively for each species, though independent, are coupled together to economize space.

It is thus seen that the majority of strains of each species must be considered to differ significantly, in a statistical sense, as regards both the mean length and also the distribution of individuals throughout the range of measurements. However, from the systematic point of view, the strains of each species must be classed together in one group because there is no real break in the variation of length of the individuals between one strain and another (cf. Tables I and II).

TABLE V.

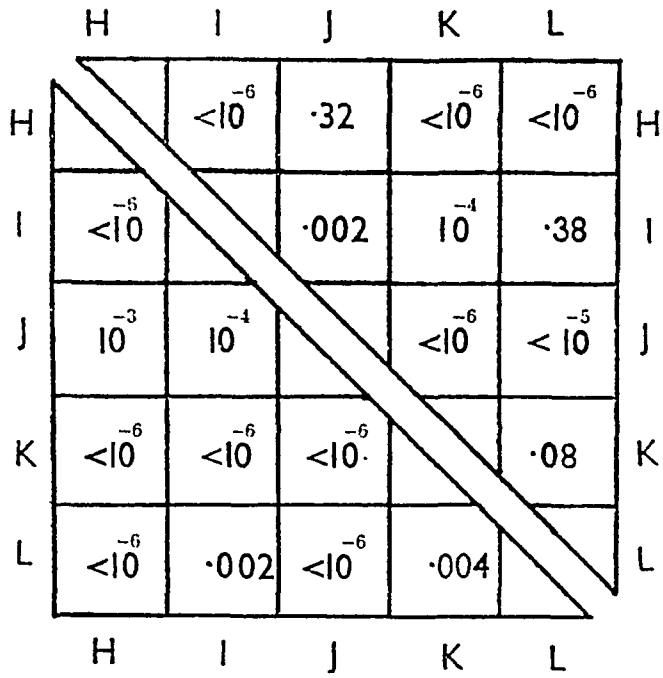


TABLE VI.

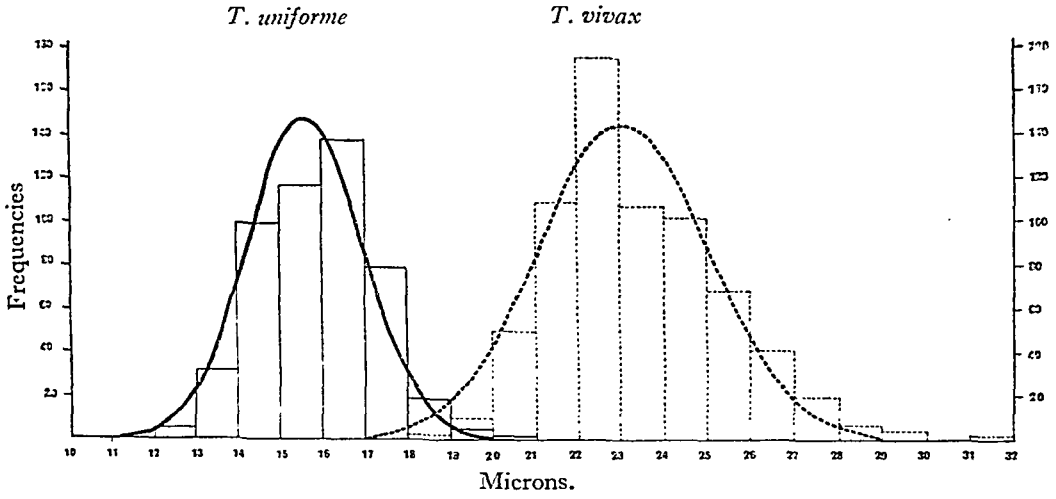
TABLES V and VI.—The probability that the observed differences in the frequency distributions in respect of length (Table V) and the differences of the mean lengths (Table VI) of the strains of *T. uniforme* could be the result of chance.

Since the different strains of each species cannot be looked upon as samples of a single population it is impossible to combine all the series of measurements and treat them together statistically, when comparing the two species. One cannot, therefore, base a method of differentiation on the normal curves fitted to all the measurements. For purely diagrammatic purposes, however, these curves are shown in Fig. D, superimposed on the histograms of the total distributions, because they give an illustration of the independence of the two species and also indicate the area within which they overlap. The common range extends from 18μ to 21μ, but, as is shown in Table VII, from 90 to 100 per cent. of the measurements of the various strains of both species lie outside these limits, except in the case of *T. vivax* Strain F. Even in that special instance, where 39 per cent. of the measurements are within the zone of overlap, it is to be

noted that twenty-nine of these are 20μ, whereas only two individuals of the total 500 *T. uniforme* reach that length (cf. Table II).

TABLE VII.

T. vivax. Percentage of individuals *more* than 21μ long.		*T. uniforme.* Percentage of individuals *less* than 18μ long.	
Strain A:	100	Strain H:	100
„ B:	99	„ I:	99
„ C:	92	„ J:	100
„ D:	94	„ K:	97
„ E:	93	„ L:	97
„ F:	61		
„ G:	98		

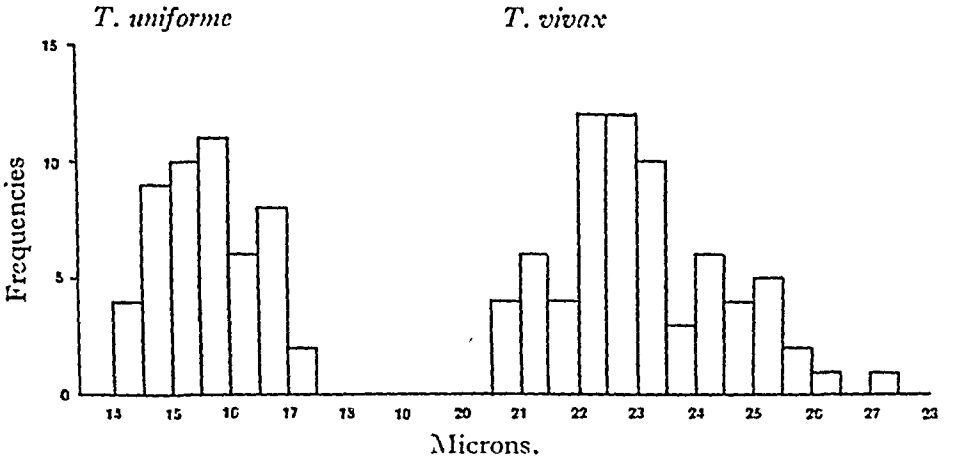


TEXT-FIG. D: Histograms illustrating the frequency distribution in respect of length, of 500 specimens of *T. uniforme* and 700 *T. vivax*, with normal curves fitted to the data superimposed. (Continuous line—*T. uniforme*; broken line—*T. vivax*.)

The overlapping of measurements among the different strains of each species was used as an argument for grouping them together. The question arises, therefore, whether one is justified in differentiating the two species on the criterion of length alone, considering that individuals of both species may fall within the range 18μ to 21μ, and that there is no other significant morphological difference between them. In our opinion this can legitimately be done because of the infrequency of these overlapping measurements. Their relative unimportance was demonstrated in the following manner: In the original series of measurements the trypanosomes were drawn as they appeared in the

film and therefore they constituted true random samples. Each series was divided up into groups of ten in the order in which they had been measured. The mean of each group was calculated, giving ten means for each strain, and a total of seventy means of ten measurements for the strains of *T. vivax* and fifty means for the *T. uniforme* strains.

The means of these small groups varied, of course, more than the means of 100, the ranges being from 14·07μ to 17·28μ for *T. uniforme* and from 20·75μ to 27·30μ for *T. vivax*. The frequency distribution of these means, grouped into classes of 0·5μ, is shown in Fig. E. It is seen that the means of each



TEXT-FIG. E : Histograms illustrating the frequency distribution of 50 means of *T. uniforme* and of 70 means of *T. vivax*, each mean obtained from a group of 10 measurements.

species form a continuous group, but that there is a considerable gap between the two. In no case was the mean of ten measurements of *T. uniforme* more than 17·5μ or the mean of ten *T. vivax* less than 20·5μ.*

Judging by these results it should, therefore, be possible in practically all cases to differentiate these two species by measuring the lengths of only ten trypanosomes. If the mean length is less than 18μ the infection is *T. uniforme*; if greater than 20μ it is *T. vivax*. In the unlikely event of the mean lying within these limits a larger series of measurements must be made to determine whether one is dealing with a sample of unusually long *T. uniforme* or unusually short *T. vivax*. In the former case, additional measurements should reduce the mean, and in the latter should increase it.

2. SYSTEMATICS.

In the foregoing statistical analysis it has been established that the two sets of strains referred to *T. vivax* and *T. uniforme* respectively actually fall into two distinct groups, which correspond to these species and are characterized

*As was shown on p. 526, similar results were obtained with the Tanganyika strains of *T. vivax* (M and N) measured by this method.

by differences in the mean lengths and in the range of lengths of the trypanosomes. Though these measurements overlap, the degree to which they do so is negligible, since from 90 to 100 per cent. of the measurements in both species lie outside the zone of overlap. The diversity of the two groups is further demonstrated by the fact that the measurement of ten randomly selected representatives of each is sufficient to differentiate them.

We now turn to the bearing of these data upon the systematic position of the two groups of trypanosomes under consideration. In the present investigation the difference in length, which originally served as the criterion for the recognition of *T. uniforme* as a species independent of the allied *T. vivax*, has been fully confirmed, extended, and established as a constant character. There can, therefore, remain no doubt that these two forms represent independent and easily distinguishable systematic units.

As was shown in Section II (p. 521 *et seq.*) the controversy regarding the relationship between *T. vivax* and *T. uniforme* was concerned, on the one hand, with the very existence of *T. uniforme* as a distinct parasite, which was challenged; and, on the other hand, with the precise systematic position of this trypanosome. The first part of the problem has now been solved, and the position of *T. uniforme*, as a parasite distinct from *T. vivax*, firmly established. As regards the exact systematic status of *T. uniforme*, the choice lies between retaining it as an independent species or assigning it to *T. vivax* with a subordinate rank.

It is usual to separate allied groups of animals as *species*, when the structural characters distinguishing them do not intergrade, i.e., when there is a morphological gap between the groups and intermediate or transitional forms are absent. If one group intergrades with another group, from which it differs in certain constant morphological features, i.e., when the two are connected by transitional forms, they are regarded as *subspecies* (or races) of the same *species*. Systematic units of lower grades, being distinguishable by inconstant characters, need not be considered here.

Since the measurements of length, which distinguish *T. uniforme* from *T. vivax*, slightly overlap (cf. Fig. D), i.e., the two are connected by transitional forms, it would seem that *T. uniforme* may be regarded as a subspecies of *T. vivax*, in which case they should be designated as *T. vivax uniforme* and *T. vivax vivax* respectively. However justifiable this course may be on formal grounds it is undesirable for several reasons. One of these is that, although the systematic units within the various subdivisions of the animal kingdom are not equivalent, they should at least have the same value within the limits of a given group, such as genus. In other words, the degree of distinction between the species of the same genus should be of the same order (cf. HOARE, 1936a). Within the genus *Trypanosoma* specific rank has in many cases been given to forms morphologically indistinguishable (e.g., in the *brucei* and *evansi* groups) or intergrading (e.g., in the *congolense* group). These and other similar forms

stand in need of revision and readjustment, but until this is done it would be inconsistent to unite *T. uniforme* with *T. vivax* as subspecies, while leaving other, more glaring, cases unchanged. Moreover, too much importance cannot be attached to the slight degree of intergradation of the lengths of these two trypanosomes, the insignificance of which was brought out in the preceding section (cf. also Table VII and Fig. E).

The most important result of the present investigation was the demonstration that *T. uniforme* and *T. vivax* are readily distinguishable by a constant character, viz., difference in length. For the sake of uniformity in the classification of trypanosomes and for other reasons given above, we propose to treat this difference as a specific character and to regard *T. uniforme* and *T. vivax* as separate species, thus retaining their original status.

VII.—Summary.

The object of this study was to define the relationship between *Trypanosoma uniforme* and *T. vivax*, and to provide exact methods for their differentiation.

The investigation was carried out by statistical methods and was based on several strains of each species from different localities. It was established that the strains corresponding to the two species fall into two distinct groups which are characterized constantly by differences in the mean lengths and in the range of lengths of the trypanosomes. It was also demonstrated that the measurement of ten individuals selected at random from any strain provides a satisfactory method for the identification of the species to which the trypanosomes belong.

The systematic position of the two groups of trypanosomes is discussed, and it is concluded that their original status as separate species, *T. uniforme* and *T. vivax*, should be retained.

A review is given of the previous records of *T. uniforme*, together with a list of hosts and the geographical distribution of this trypanosome.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

A NOTE ON THE TYPES OF PNEUMOCOCCI PREVALENT IN LAGOS.

BY

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Nigeria.

During the period extending from the end of August, 1936, until June, 1937, an investigation of the types of pneumococci prevalent in Lagos was commenced. Lobar pneumonia became mildly epidemic in Lagos at the end of 1936 and in the beginning of 1937.

METHODS OF INVESTIGATION.

A clinical examination was made, whenever possible, in every case of suspected pneumonia, and details of the duration of the illness, age, sex, etc., were obtained. A blood culture was made, and a specimen of sputum was collected at the same time. If, on bacteriological examination, the sputum showed Gram-positive diplococci suggestive of pneumococci, the material was emulsified and inoculated intraperitoneally into white mice. On the death of these animals, some 12 hours later, cultures were made on blood or chocolate agar from the peritoneal exudate and from the heart blood.

When the cultural appearances and the morphology of the organism were typical, the bile solubility test and the fermentation of inulin were proved in each case.

An agglutinating serum was prepared against the first strain of pneumococcus isolated (Table I), by the method described by COLE and MOORE*. According

* COLE, RUFUS, & MOORE, H. F. (1917). *J. exp. Med.*, 26, 537.

TABLE I.

Strain.	Name.	Sex.	Age.	Day of Illness.	Blood Culture.	Sputum.	Type.	Remarks.
1	J.D.	M.	58	10th	Negative	Positive	Group IV	Agglutinating serum prepared.
2	A.O.	M.	20	7th	„	„	„	
3	O.D.	M.	35	3rd	„	„	„	
4	P.M.	M.	45	6th	Positive	„	„	
5	D.A.	M.	17	5th	Negative	„	„	
6	D.L.	M.	28	4th	„	„	„	
–	G.L.	M.	36	?	„	„	„	Avirulent for mice.
–	M.O.	M.	40	9th	„	„	—	„ „
–	A.L.	M.	20	4th	„	„	—	„ „
–	V.	M.	40	4th	„	„	—	Virulent for mice. Patient died of acute appendicitis —no pneumonia.

to this method, doses of 0·5 c.cm. of an emulsion obtained by re-suspending the centrifuged deposit from a measured volume of a 24-hour growth of the organism in half the amount of normal saline, after the organisms had been killed by heat, were inoculated intravenously into a rabbit. The inoculations were given every day for 6 days, followed by 1 week of rest, over a period of 6 weeks. Although this was rather a troublesome and lengthy method, a good agglutinating serum with a titre of 1 in 80 was obtained. (The agglutination of the rabbit's serum to the homologous organism before the inoculations were commenced was 1 in $2\frac{1}{2}$, doubtful.)

The first six strains of pneumococci obtained were tested against this serum; the results were inconclusive—complete agglutination did not take place in any tube, and partial agglutination was noted in the low dilutions only. Suspensions of these strains were preserved, and were tested later against standard Type Sera I, II and III. It was found that all six strains failed to agglutinate with any of these. Unfortunately it was not possible to submit them to further typing. The first six strains isolated (Table I) were therefore considered to be Group IV pneumococci; it is to be noted that only one of these was obtained by blood culture. In three other cases examined at this time, the sputum showed diplococci suggestive of pneumococci, but emulsions of the sputum proved avirulent for mice. One case with virulent pneumococci in the sputum died of appendicitis and not pneumonia.

In order to be certain that the pneumococci investigated were actually pathogenic, it was decided in December, 1936, to work only with strains obtained by blood culture. Doubt as to the pathogenicity of strains obtained from sputa

was confirmed by the isolation of a virulent pneumococcus from the sputum of a patient who later, as ascertained at postmortem examination, died from acute appendicitis, without any gross lung lesion.

In establishing the identity of pneumococci isolated in this way, the same criteria as before were required, *viz*: typical morphology and cultural appearances, with positive bile solubility test and the ability to ferment inulin, with the production of acid.

The viability of the strains was maintained by frequent subculture, and nine strains were identified (Table II). Two strains isolated at this time (Strains 9 and 10, Table II) did not survive.

Agglutinating sera were prepared for two strains selected at random (Strains 8 and 13, Table II), using the same method as was adopted for Strain 1, during

TABLE II.

Strain.	Name.	Sex.	Age.	Day of Illness.	Blood Culture.	Type.	Remarks.
7	J.O.	M.	29	4th	Positive	2	
8	G.M.	M.	32	1st	"	5	
9	J.S.	M.	36	9th	"	Group IV	
10	N.T.I.J.	M.	20	3rd	"	"	Strain died before final typing possible
11	A.A.	M.	25	5th	"	5	Strain died before final typing possible
12	T.I.	M.	24	5th	"	12	
13	M.J.	M.	35	7th	"	5	
14	G.A.	M.	26	2nd	"	12	
15	J.R.	M.	26	15th(?)	"	?	
16	M.	M.	33	5th	"	12	Subculture failed.
17	J.J.	M.	36	6th	"	11	
18	G.N.	M.	?	?	"	12	
19	A.A.	M.	26	5th	"	?	Subculture failed.

the period which elapsed before the final typing was possible. It was unfortunate that both these strains were later found to belong to the same type (Type V pneumococcus).

In this second series of investigations, twenty-nine blood cultures in all were made, and of these thirteen were positive, with the findings indicated in Table II. During the investigation, an attempt was made to preserve the viability of pneumococci by drying portions of mouse heart in cases where the animals had died of the infection, but it was found that the pneumococci did not survive for any lengthy period. Drying *in vacuo* might have been more efficacious.

Summary and Conclusions.

In the first series of cases, six virulent strains of pneumococci were isolated (one by blood culture). All these were found to belong to Group IV. Three cases in which diplococci morphologically suggestive of pneumococci occurred in the sputum were found to be avirulent for mice. One case with virulent pneumococci in the sputum died of acute appendicitis with no gross lung lesion.

In the second series, twenty-nine blood cultures were made, of which thirteen were positive for pneumococci and were identified as shown in Table II. Two strains died before final typing could be carried out, but these were not Type I, II or III. Two strains failed to grow on subculture from the original blood cultures. Sixteen blood cultures were negative.

This short and admittedly incomplete investigation unfortunately had to be discontinued for lack of time. The findings would seem to show that the pneumococci prevalent in Lagos during the period under consideration were mainly types included under "Group IV pneumococcus." Only one Type II pneumococcus was isolated during the investigation, and it would be interesting to know if such a finding is representative: no conclusion can of course be drawn without knowing the findings in a larger number of cases. If the results were confirmed, the question of the use of Type I or Type II therapeutic serum would not arise.

I am indebted to Dr. R. Briercliffe, Director of Medical Services, Nigeria, for permission to publish, and to Dr. J. A. Young for his assistance and guidance in this work, and for his kindness in arranging that Strains 8, 11, 12, 13, 14, 16, 17 and 18 were sent to Dr. F. Griffith, Ministry of Health Laboratories, London, who was good enough to undertake the final typing.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

HAEMOGLOBINAEMIA IN MALARIA.

BY

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Before the work of BARRATT and YORKE in 1909, it was apparently assumed that malaria must be associated with haemoglobinaemia. Those observers attempted quantitative estimation of plasma haemoglobin upon finger blood from fourteen cases of malaria, seventeen cases of blackwater fever, and some controls, without finding significant increase in malaria.

YORKE, MURGATROYD and OWEN (1930), and FAIRLEY and BROMFIELD (1933) improved the technique for quantitative estimation.

FAIRLEY and BROMFIELD (1933), in their conclusions, stated that malaria is unaccompanied by haemoglobinaemia, but foresaw, in their comment upon the results obtained in their study, that it might be found in cases of "primary hyper-infection producing severe anaemia and intense jaundice, such as one occasionally encounters in the tropics." Dr. FAIRLEY also expressed himself to

us as believing that this is a subject well worthy of investigation under tropical conditions.

In the course of work upon the aetiology of blackwater fever we have examined, with controls, a number of cases of malignant tertian malaria with respect to the haemoglobin content of the " true " plasma.

TECHNIQUE.

The technique employed has been, essentially, that of FAIRLEY and BROMFIELD (1933). Particularly in hot weather we have regarded it as important, in the collection of blood under sterile liquid paraffin, to avoid strong suction with the syringe, or great pressure in introducing the blood below the paraffin in the centrifuge tube; and, when the sample is in the tube, only gentle inversion, with rapid rotation, is permissible for mixing with the neutral oxalate.

The spectroscopic method for quantitative estimation of blood pigments is practically that of BLOEM (1933)—long used in general chemical procedure—but a standard glass cell having optically parallel sides and giving a thickness of column viewed of 13 mm. is used. We arranged a wood block to hold the spectroscope and glass cell in front of the microscope lamp.

To determine the sensitivity of the apparatus for oxyhaemoglobin and methaemoglobin, the oxygen capacities of eight samples of oxalated blood were determined by treatment of the fully aerated blood in the Van Slyke volumetric gas apparatus, and thence the haemoglobin content calculated, as grammes per 100 c.c., by multiplying by 0·746.

(*a*) *Oxyhaemoglobin.*

Of each of the bloods an accurately measured quantity (0·2 c.c.) was diluted with distilled water until the α band of the spectrum just disappeared, and so the dilution to which any one volume of that blood could be so diluted, was arrived at. (Averages of at least two trial dilutions were taken in each case, and then the average taken for the eight blood samples.) Division of the haemoglobin content by the dilution factor gives the lowest concentration of oxyhaemoglobin detectable with the particular apparatus.

We have adhered to expression of concentrations in mg. per 100 c.c. rather than in percentages of any standard because the majority of biochemical estimations are commonly so recorded and because the standard of Haldane (oxygen capacity 18·5 vols. per cent., Hb., 13·8 grammes per 100 c.c.) hardly represents the average for any large number of healthy adults at high altitude in the tropics (altitude here is 3,800 feet above sea level).

(*b*) *Methaemoglobin.*

A further sample of each of the oxalated bloods was used for production of methaemoglobin by the method of MCELLROY (1920), and after standing for 5 minutes, was diluted and observed as for oxyhaemoglobin..

We concluded that our apparatus is sensitive to amounts of oxyhaemoglobin at least as small as 5 mg. per 100 c.c. of solution, and to amounts of methaemoglobin at least as small as 100 mg. per 100 c.c. of solution.

RESULTS.

Apart from cases of blackwater fever, we have, so far, examined in this way "true" plasma from seventeen cases of malignant tertian malaria, and from twenty-five cases other than malarial.

Control Cases.

Each was checked, apart from symptoms, by absence of parasites in the blood. None had methaemoglobinaemia, and only in nine was oxyhaemoglobinaemia detected. The concentration figures were between 5 and 15 mg. per 100 c.c. of plasma.

Malarial Cases.

All showed *Plasmodium falciparum* in thick or thin blood films—infections of varying intensity. Some were relapse cases, but certainly the majority were re-infections: a few were primary infections. Nine showed no haemoglobin in the plasma, four showed traces (5 to 15 mg. per 100 c.c.), and the remaining four showed, as highest figures, 60, 42·5, 35 and 22·5 mg. per 100 c.c. respectively. In no case was methaemoglobinaemia observed.

Two of the last group of cases mentioned had normal or subnormal temperature when the oxyhaemoglobinaemia was detected.

We are not aware that concentrations such as those in these last cases have been recorded in malaria (FAIRLEY and BROMFIELD noted 16·1 mg.) and accordingly present some further particulars of our cases.

CASE I.

V. de W., a young man of 22 who came for advice, being apprehensive about blackwater fever and having an uncomfortable sensation for some days about his heart. He had had two recrudescences of old "fever" in the preceding 5 or 6 weeks, and he felt ill again now (as he appeared to be). In the same state for the last 5 days, he had been using small irregular doses of quinine.

His temperature was subnormal, pulse very slow, blood pressure low.

His liver and spleen were slightly enlarged and tender, his conjunctivae "putty coloured."

The urine was very acid, and contained diacetic acid, urobilin and excess of protein. It was scanty in amount (690 c.c. in first 24 hours).

A few tube casts were found in the deposit—culture of one specimen showed it to be sterile.

The blood films showed a moderate infection with *P. falciparum*, and active phagocytosis of erythrocytes.

As to the slow pulse, we were not able, on the second day of attendance, when the rate was 56 per minute, to demonstrate with the polygraph any A.V. block. On the 3rd day the pulse rate was normal and blood pressure 132/62. Capillary pulsation was then visible in the nail beds.

Biochemical and other particulars are set out in Table I (page 542).

TABLE I.

Case I. V. de W.	Day of Illness. 7th (approximately).	8th.
Blood		
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	86	80
Erythrocytes—millions per c.mm.	4·68	6·3
Leucocytes per c.mm.	15,687	14,375
Film examination	Fairly numerous M.T. parasites. Phagocytosis of R.B.C.'s noted	
True Plasma		
Pigments—		
Methaemoglobin	Nil	Nil
Oxyhaemoglobin—mg. per 100 c.c.	60	30
Bilirubin. Van den Bergh Reaction		
Direct	Negative	Negative
Indirect	2 units	0·5 unit
CO_2 combining power. Vols. per cent.	57·7	60
Inorganic phosphates: mg. per 100 c.c.	4·7	2·6
Phosphatase: Jenner and Kay units	36	33·6
Serum		
Calcium: mg. per 100 c.c.	13	10·8
Lipase: 0·5 c.c. liberates fatty acids equivalent to $N/_{10}$ NaOH	0·21 c.c.	0·1 c.c.
Free haemolysin	Nil	Nil
Whole Blood. Group O		
Uric acid: mg. per 100 c.c.	4·54	3·3
Urea: mg. per 100 c.c.	60	38
Amino-acid N.: mg. per 100 c.c.	5	4
Cholesterol ,, ,,	220	226
,, erythrocytes ,,	247	271
Lecithin	545	557
,, in erythrocytes ,, ,,	646	
Lipin P.	21·7	22·7
,, in erythrocytes ,, ,,	27·7	
Blood Pressure. mm. Hg.		
Systolic	104	110
Diastolic	70	60
Pulse Rate, per minute	50	56
Urine		
pH	Less than 3·5	
Ketones	Diacetic acid present	
Reducing substances	Nil	
Albumin	Distinct trace	
Pigments—		
Met- and Oxy-haemoglobin	Nil	
Urobilin	Present	
Quantity	Small	
Collected and measured from noon on 8th day onwards		P.M., 12.15, 130 c.c.; 3, 240 c.c.; 8, 100 c.c. A.M., 6, 220 c.c.; 9, 500 c.c.; 11, 400 c.c. *i.e.*, 690 c.c. in 21 hours, but 900 c.c. in next 3 hours, so total 1,590 c.c. in 24 hours
Deposit—only occasional tube casts seen. No pus, R.B.C.'s or other abnormality.		
Culture—on one occasion—sterile		

CASE II.—E.P., a man of 40, who had no major attack of malaria for 9 years. Blood films showed a very heavy M.T. infection—many erythrocytes containing two "ring" forms, and numerous extracellular parasites were seen. There was some hyperpyrexia, and the temperature was above 102° F. during the greater part of 3 days. Great restlessness, severe headache and loin pains were features of the attack. The considerable blood destruction (*vide* Table II) did not produce much jaundice. The pulse was slow (54 to 56 per minute) on the 2nd and 3rd days—before any jaundice showed—and normal on the 4th day. Praecordial distress was marked just before the heart rate became reduced.

TABLE II.

Case II. E.P.	Day of Illness.			
	1st.	2nd.	3rd.	4th.
Blood				
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	100	110		86
Erythrocytes: millions per c.mm.	6·2		4·8	4·31
Leucocytes per c.mm.	4,000	5,937		
Film examination	Very heavy M.T.infection. Many cells, two rings in a cell, and extracellular parasites seen			Parasites present
True Plasma				
Pigments—				
Methaemoglobin		Nil		
Oxyhaemoglobin: mg. per 100 c.c.		22·5		
Bilirubin: Van den Bergh Reaction				
Direct		Negative		
Indirect		2 units		
Inorganic phosphate: mg. per 100 c.c.		2·9		
CO_2 combining power. Vols. per cent.		70		
Phosphatase: Jenner and Kay units		4·54		
Whole Blood				
Uric acid: mg. per 100 c.c.		2·38		
Urea: mg. per 100 c.c.		61·5		53·3
Amino-acid N: mg. per 100 c.c.		6·3		
Blood Pressure: mm. Hg.				
Systolic	124	130		126
Diastolic	80	86		90
Pulse Rate per minute	110	54	56	74
Urine				
pH		About 3		
Ketones		Nil		
Reducing substances		Nil		
Protein: per cent.		0·03	0·028	
Pigments: Met- and oxyhaemoglobin		Nil		
Urobilin		Present		
Quantity: 24 hours			580 c.c.	1,300 c.c.
Deposit		Numerous epithelial and granular tube casts		Casts scanty

TABLE I.

Case I. V. de W.	Day of Illness.	
	7th (approximately).	8th.
Blood		
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	86	80
Erythrocytes—millions per c.mm.	4·68	6·3
Leucocytes per c.mm.	15,687	14,375
Film examination	Fairly numerous M.T. parasites. Phagocytosis of R.B.C.'s noted	
True Plasma		
Pigments—		
Methaemoglobin	Nil	Nil
Oxyhaemoglobin—mg. per 100 c.c.	60	30
Bilirubin. Van den Bergh Reaction		
Direct	Negative	Negative
Indirect	2 units	0·5 unit
CO_2 combining power. Vols. per cent.	57·7	60
Inorganic phosphates: mg. per 100 c.c.	4·7	2·6
Phosphatase: Jenner and Kay units	36	33·6
Serum		
Calcium: mg. per 100 c.c.	13	10·8
Lipase: 0·5 c.c. liberates fatty acids equivalent to $N/_{10}$ NaOH	0·21 c.c.	0·1 c.c.
Free haemolysin	Nil	Nil
Whole Blood. Group O		
Uric acid: mg. per 100 c.c.	4·54	3·3
Urea: mg. per 100 c.c.	60	38
Amino-acid N.: mg. per 100 c.c.	5	4
Cholesterol ,, ,,	220	226
,, erythrocytes ,,	247	271
Lecithin	545	557
,, in erythrocytes ,, ,,	646	
Lipin P.	21·7	22·7
,, in erythrocytes ,, ,,	27·7	
Blood Pressure. mm. Hg.		
Systolic	104	110
Diastolic	70	60
Pulse Rate, per minute	50	56
Urine		
pH	Less than 3·5	
Ketones	Diacetic acid present	
Reducing substances	Nil	
Albumin	Distinct trace	
Pigments—		
Met- and Oxy-haemoglobin	Nil	
Urobilin	Present	
Quantity	Small	
Collected and measured from noon on 8th day onwards		P.M., 12.15, 130 c.c.; 3, 240 c.c.; 8, 100 c.c. A.M., 6, 220 c.c.; 9, 500 c.c.; 11, 400 c.c. *i.e.*, 690 c.c. in 21 hours, but 900 c.c. in next 3 hours, so total 1,590 c.c. in 24 hours
Deposit—only occasional tube casts seen. No pus, R.B.C.'s or other abnormality.		
Culture—on one occasion—sterile		

CASE II.—E.P., a man of 40, who had no major attack of malaria for 9 years. Blood films showed a very heavy M.T. infection—many erythrocytes containing two "ring" forms, and numerous extracellular parasites were seen. There was some hyperpyrexia, and the temperature was above 102° F. during the greater part of 3 days. Great restlessness, severe headache and loin pains were features of the attack. The considerable blood destruction (*vide* Table II) did not produce much jaundice. The pulse was slow (54 to 56 per minute) on the 2nd and 3rd days—before any jaundice showed—and normal on the 4th day. Praecordial distress was marked just before the heart rate became reduced.

TABLE II.

Case II. E.P.	Day of Illness.			
	1st.	2nd.	3rd.	4th.
Blood				
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	100	110		86
Erythrocytes: millions per c.mm.	6·2		4·8	4·31
Leucocytes per c.mm.	4,000	5,037		
Film examination	Very heavy M.T. infection. Many cells, two rings in a cell, and extracellular parasites seen			Parasites present
True Plasma				
Pigments—				
Methaemoglobin		Nil		
Oxyhaemoglobin: mg. per 100 c.c.		22·5		
Bilirubin: Van den Bergh Reaction		Negative		
Direct		2 units		
Indirect		2·9		
Inorganic phosphate: mg. per 100 c.c.		70		
CO_2 combining power. Vols. per cent.		4·54		
Phosphatase: Jenner and Kay units		2·38		
Whole Blood		61·5		
Uric acid: mg. per 100 c.c.		6·3		53·3
Urea: mg. per 100 c.c.	124			
Amino-acid N: mg. per 100 c.c.	80	130		
Blood Pressure: mm. Hg.	110	86		126
Systolic		54		90
Diastolic			56	74
Pulse Rate per minute		About 3		
Urine		Nil		
pH		Nil		
Ketones		0·63		
Reducing substances		Nil	0·028	
Protein: per cent.		Present		
Pigments: Met- and oxyhaemoglobin				
Urobilin				
Quantity: 24 hours		580 c.c.	1,300 c.c.	
Deposit		Numerous epithelial and granular tube casts	Casts scanty	

Case III.

Mrs. W. E., aged 58, a woman said to be experiencing a primary attack of malaria. she was new to the tropics and very frightened and nervous.

The blood showed a heavy M.T. infection, which responded promptly to specific treatment without development of anaemia or icterus.

Table III.

Case III. Mrs. W. E.	Day of Illness.		
	2nd.	3rd.	4th.
Blood			
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	92		110
Erythrocytes : millions per c.mm.	5·8		
Reticulocytes, per cent.	1		
Leucocytes, per c.mm.	7,024		
Film examination	Heavy M. T. infection		
True Plasma			
Pigments—			
Methaemoglobin	Nil		
Oxyhaemoglobin : mg. per 100 c.c.	35		
Bilirubin : Van den Bergh Reaction			
Direct	Negative		
Indirect	2·2 units		
Inorganic phosphate : mg. per 100 c.c.	3·4		
CO_2 combining power. Vols. per cent.	64·3		
Phosphatase : Jenner and Kay			
Whole Blood			
Uric acid : mg. per 100 c.c.	3		
Urea : mg. per 100 c.c.	42	44·4	36
Urine			
pH	About 5	6·5	7
Ketones	Nil	Nil	Nil
Reducing substances	,,	,,	,,
Protein : per cent.	0·03	0·036	0·028
Pigments : Met- and oxyhaemoglobin	Nil	Nil	
Urobilin	1 c.c. diluted to 2·5 shows spectrum	1 c.c. may be diluted to 4·55 c.c.	
Deposit		Numerous hyaline and granular casts, no pus or R.B.C.'s	Fewer casts

CASE IV.—McL., a man of 35, who had not thought that he might be suffering from any sort of malaria, but who had a pain in his left side and had, for many weeks, felt so ill and weak that he was beginning to despair about being able to continue at his work—in a store. He appeared pale, and the whites of his eyes were not clear, but the blood showed no anaemia. The heart sounds were normal—blood pressures low. There were a few carious teeth, injected fauces, a few crepitations near the angle of the left scapula (a history of old pneumonia on the opposite side) and a normal blood sedimentation rate. To our surprise, *P. falciparum* and pigmented mononuclears were discovered in the blood, and oxyhaemoglobin in the "true" plasma. His temperature was then recorded as raised. His urine was essentially normal. Given specific treatment he soon lost his pain, and then the weakness, and his whole general condition improved dramatically. Other details are given in Table IV.

TABLE IV.

Case IV. McL.	Day of Examination.	
	1st.	17th.
Blood		
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	90	94
Erythrocytes: millions per c.mm.	6·85	6·89
Leucocytes: per c.mm.	7,500	6,875
Film examination	Scanty M.T. parasites, curious marbling of R.B.C.'s, occasional pigmented mononuclears	
True Plasma		
Pigments—		
Methaemoglobin	Nil	
Oxyhaemoglobin: mg. per 100 c.c.	42·5	Nil
Bilirubin: Van den Bergh Reaction		
Direct	Negative	
Indirect	0·5 units	
CO_2 combining power. Vols. per cent.	78	20
Inorganic phosphate: mg. per 100 c.c.	5	Less than 0·5 units
Phosphatase: Jenner and Kay units	7	75·8
Whole Blood		
Urea: mg. per 100 c.c.	34	2·58
Cholesterol: mg. per 100 c.c.	214·8	3
" (cells) " "	197·3	38·1
Lecithin: mg. per 100 c.c.		220·5
" (cells) " "		168·1
Cell sedimentation rate (Zeckwer and Goodell-modif.) at 1 hour cells at	835·6	
Urine		
pH	9·2 line	
Ketones		
Reducing substances	About 6·5	
Protein	Nil	
Pigments: Met- and oxyhaemoglobin	"	
Urobilin	No increase	Nil abnormal
Deposit	Nil	
	"	
	Nil abnormal	

Two of the oxalated blood samples examined in connection with Cases I–IV. showing oxyhaemoglobinaemia were transported by road before being studied in the laboratory.

These were the second sample taken in Case I (V. de W.) and the one from Case II (E.P.). The first of these was carried 2½ miles over a fair road; and will be seen to show less oxyhaemoglobin in the plasma than the first sample taken in the case which was examined at once without being transported at all; the second was carried just over 1 mile along an excellent road.

Air temperature was not high on either of these occasions.

TABLE V.

Case V. H.M.P.	Day of Illness.	
	2nd.	4th.
Blood		
Haemoglobin per cent. of 15·6 grammes per 100 c.c.	80	80
Erythrocytes: millions per c.mm.	4·15	4
Leucocytes: per c.mm.	5,312	7,500
Film examination	Heavy M.T. infection gametocytes numerous	
True Plasma		
Pigments—		
Methaemoglobin	Nil	Nil
Oxyhaemoglobin: mg. per 100 c.c.	7·5	,,
Bilirubin: Van den Bergh Reaction		
Direct	Much delayed	Negative
Indirect	2·7 units	1·75 units
CO_2 combining power. Vols. per cent.	72	
Inorganic phosphate: mg. per 100 c.c.	5	3·2
Phosphatase: Jenner and Kay units	4·68	11·58
Whole Blood		
Uric acid: mg. per 100 c.c.	4·1	3·1
Urea: mg per 100 c.c.	38	26
Amino-acid N.: mg. per 100 c.c.	5·6	4·8
Cholesterol: mg. per 100 c.c.	241·9	
,, of erythrocytes: mg. per 100 c.c.	210·6	
Lecithin: mg. per 100 c.c.	385·6	
,, of erythrocytes: mg. per 100 c.c.	455·8	
Urine		
pH	About 6	
Protein	Distinct trace	
Ketones	Nil	
Reducing substances	,,	
Bile pigments	Traces	
,, acids	Nil	
Urobilin	1 c.c. can be diluted to 16 c.c. before spectrum vanishes	

COMMENT.

In malignant tertian malaria not so much evidence is available as to appearances of parasitized erythrocytes just prior to their rupture in schizogony as in the other species, and possibly intra-corpuscular conversion of haemoglobin into pigment is not so completely brought about by *P. falciparum* as by the other plasmodia. This suggests itself as a possible partial explanation of haemoglobinaemia in some cases of M.T. malaria, but what other factors might reasonably be regarded as in any way contributory ?

Such haemoglobin as may escape in relatively unaltered condition from the disrupted cells will constitute a circulating " foreign " protein of very complex formula. (Its molecular weight is in the neighbourhood of 70,000, and it is not yet established that polymers of Hb.—perfectly discrete molecules, containing more or less iron—cannot occur in the human body.)

Probably at least four reasonably distinct functions of the liver, with complementary activity of cells of the reticulo-endothelial system, and possibly of certain renal cells, are involved in the katabolism of plasma haemoglobin ; and whatever the efficiency in a given case of the other liver functions, the " pigmentary " function may well be heavily burdened even quite early in a malarial infection.

If any of these processes be sufficiently embarrassed it is to be expected that haemoglobinaemia may result.

The case (H.M.P.) described in Table V is a typical example from the group of malarial cases which show high plasma bilirubin but no plasma haemoglobin, and we have the impression that the two substances tend to occur in inverse concentration.

Each of our few cases showing haemoglobinaemia had low blood pressures. Two of them had bradycardia—one after about 30 hours of high fever—while the other had, so far as we were able to discover, no raised temperature at all for many days before being seen, and certainly not afterwards. The third case of haemoglobinaemia also ran an apyrexial course.

Phagocytosis of parasitized erythrocytes was certainly going on actively in Case I (V. de W.), and yet unchanged haemoglobin escaped into the plasma.

No cause was discovered to explain the leucocytosis in the same case—we felt that it probably represented another aspect of the same process. UCHIDA (1921), in a detailed study of blood changes in paroxysmal haemoglobinuria, recorded leucocytosis as a constant finding within 2 hours of chilling, and attributed it to destruction of erythrocytes. He described " auto-haemotropin " in the blood of such cases as a substance promoting phagocytosis of the patient's own erythrocytes by his leucocytes.

Three of our malarial cases with haemoglobinaemia showed, at any rate temporarily, numerous tubular casts in the urine.

In two of these, Cases II and III (E.P. and W.E.), it was felt that the highest figures for urea in the blood (61·5 and 44·4 mg. per 100 c.c.) could be attributed

to increased katabolism associated with the fever, but the same could hardly hold in Cases I and IV (V. de W. and McL.).

In the first of these, although the ketosis was probably due to vomiting, there was increase of plasma inorganic phosphate as well as of nitrogenous constituents in the whole blood; and in the second the blood urea remained for more than 10 days towards the upper limit of normality and the plasma inorganic phosphate was raised at the first examination.

It would appear, from studies of blackwater fever, that the human renal threshold for haemoglobin is approached by a plasma concentration in the neighbourhood of 250 mg. per 100 c.c., but it is possible that the cells of healthy secreting tubules may eliminate small undetectable traces of the pigment and may themselves be injured in the process.

SUMMARY.

In a small series of malignant tertian infections four cases of significant oxyhaemoglobinaemia are recorded.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 5. March, 1938.

INVESTIGATION ON THE PURIFICATION OF WATER WITH RESPECT TO SCHISTOSOME CERCARIAE.

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1.—Introduction.

The existing water supply of Tel-Aviv is provided by a number of wells bored within the area of the town. As these wells will be inadequate in the near future to meet the requirements of the growing population, and also because the water itself is hard and tends to become salty, the erection of a large water-supply and water-purification plant on the river Yarkon is contemplated.

The water in the River Yarkon is adequate and from a chemical standpoint is of a much better quality than the well water, and if purified by appropriate means, could well serve the needs of the growing town of Tel-Aviv. It is reputed, however, to be contaminated with schistosome cercariae and therefore the question has arisen whether the usual methods of water purification will be adequate to remove the danger of schistosomiasis.

Since no definite conclusions could be drawn from the existing literature, it was found necessary to carry out the experiments described below.* The conclusion that we have reached is that it is possible to exterminate the schistosome cercariae by the usual methods of water purification.

2.—Present State of Water Purification in Respect to Schistosome cercariae.

Three main processes are generally used for the purification and sterilization of drinking water :—

(1) Clarification through precipitation by means of aluminium sulphate ($Al_2(SO_4)_3$) diluted in the raw water up to 100/1,000,000 ; (2) Filtration through a filtering layer, such as sand, this being mostly used in connection with the former process ; (3) Sterilization by means of gaseous chlorine or by chlorine compounds which contain active chlorine. Generally solutions up to 1/1,000,000 active chlorine are used, if stronger solutions are applied the surplus is eliminated after the treatment by the so-called " dechlorination process." Besides chlorination, sterilization by means of ozone, ultra-violet rays, etc., is also used.

There exists a rather extensive literature on the sterilization value of all these processes as far as bacteria are concerned. However, few data are published regarding the effectiveness of these processes on the cercariae of *Schistosoma*. These data are often contradictory and therefore they do not give a clear ideas as

* This investigation was carried out thanks to a grant offered by the Municipality of Tel-Aviv.

Thanks are due to Dr. Abdel Azim and his staff of the Endemic Diseases Hospital, Cairo, as well as to Dr. Rameses Girges, Tanta, for their kind and suggestive help in obtaining infected snails. We wish to thank Mr. Morris, of the Cairo Water Co., and to Mr. Walton, of the Alexandria Water Co., for their readiness to supply us with the necessary information concerning the purification of water in Egypt.

to the reliability of the usual sterilization methods for the destruction of these cercariae.

The following is a discussion of the more important data :—

(a) Gaseous Chlorine.

THRESH *et al.* (1933, p. 568) write that the cercariae are not destroyed by chlorine in concentrations usually employed in the waterworks (up to two parts per million).

RAMESES GIRGES (1934, p. 519) writes : " It was shown that chlorine in a concentration of 1/1,000,000, usually employed in hot climates to sterilize water, has no effect on living cercariae. . . ."

(b) Calcium Hypochlorite (bleaching powder solution).

According to LEIPER (1916, p. 179) a freshly prepared solution of bleaching powder destroys the cercariae immediately in concentration of 1/30,000 (= 33 per million) ; after 3 minutes in concentration of 1/50,000 (= 20 per million), and does not kill them at all, even after expiration of 1¼ hours, in a concentration of 1/300,000 (= 3·3/1,000,000) of available chlorine.

MANSON-BAHR and FAIRLEY (1920, p. 67) state : " The commonest bactericide utilized in the Army is the bleaching powder, one part of available chlorine per 1,000,000 parts of water being considered efficient. In the small book published by the War Office (*Memorandum on Some Medical Diseases in the Mediterranean War Area*) is stated that one part per million of available chlorine is efficient in killing cercariae. In numerous experiments we have proved the fallaciousness of this statement. Thus, after 2½ hours' immersion in water containing four parts per 1,000,000 available chlorine, we have found cercariae alive and very motile. The cercariae were obtained from *Planorbis boissyi* and the bleaching powder used contained 28 per cent. available chlorine."

BLACKMORE (1928) made experiments with *S. mansoni* cercariae in tap water, unclarified Nile water and aquarium water. On p. 363 he states : " In all three types of water on addition of bleach to give the amount of chlorine necessary for destruction of pathogenic bacteria, the cercariae become motionless in from 5 to 15 minutes. . . ."

According to the experiments of GRIFFITH-JONES and his collaborators (1930) bleaching powder solution containing five per million available chlorine kills both species of cercariae within 10 to 15 minutes, while a solution of one per million is not certain in killing them within 2½ hours.

(c) Chloramine.

BLACKMORE (1928, p. 262) observed that in chloramine solution " prepared according to the instructions of the monochloramine method " and containing one per million available chlorine the cercariae were killed in about 5 minutes.

According to GRIFFITH-JONES *et al.* (1930) : " Chloramine, when prepared in the apparatus supplied by Messrs. United Water Softeners Ltd., and in accordance with the printed instructions which they supply, is less effective than chlorine in killing the cercariae. Chloramine, when prepared according to the ' water cart method ' is more effective in killing the cercariae than bleaching powder." According to the experiments of these authors the solution of chloramine prepared by the former method and containing six per million available chlorine kills the cercariae of *S. mansoni* within 20 minutes, two per million within 2½ hours, and one per million does not kill all the cercariae even within 3½ hours. A solution of chloramine prepared according to the " water cart method " and containing two per million available chlorine kills both species of cercariae within 15 minutes, containing one per million chlorine—within 30 to 60 minutes and in concentration of 0·8 per million it kills only a proportion of the cercariae after 3½ hours.

According to KHALIL (1934) chloramine produced by mixing a patented compound " Chlorosene T " with ammonium chloride killed the schistosome cercariae in purified

water within 30 minutes in a solution containing one per million of available chlorine and within 1 hour when ordinary Nile water was used.

In the *Army Manual of Hygiene and Sanitation* (1934) the following statement is made on p. 184 :

" Ordinary chlorination of drinking water cannot be relied on and at least twice the normal amount of water sterilising powder must be used. After preliminary clarification cercariae can be killed and water rendered safe for drinking in 1 hour by treatment with a standard dosage of chloramine. The standard dose for 100 gallons of water, for use 1 hour after treatment, is two tablets of ammonium chloride followed by two scoopfuls of chlorosene. By doubling the standard dosage cercariae in clarified water are killed in half an hour."

(*d*) *Lime-Water.*

RUGE *et al.* (1925, p. 346) write " the addition of quick-lime into infected water protects the experimental animals against infection." THRESH *et al.* (1935, p. 568) state : " There is evidence that they (the cercariae) are killed by excess-lime treatment of water." According to RAMESES GIRGES (1934, p. 519) " Lime solution in a proportion of 1 : 1,000 kills cercariae in 30 minutes."

According to KAN (1934), lime-water 1 in 2,000 instantaneously kills the free swimming cercariae of *S. japonicum*.

(*e*) *Aluminium Sulphate* (*Alum*).

RAMESES GIRGES (1934, p. 520, citing from LEIPER) writes that " Alum, in the dilution used for precipitation of canal water has no effect on the cercariae. The flocculent precipitate of alum does not hold the cercariae which are seen actively swimming in the solution 12 hours after the addition of the alum."

THRESH and others (1933) state that the cercariae cannot be completely removed by coagulants.

(*f*) *Filtration.*

According to RAMESES GIRGES, p. 520 (citing from LEIPER) : " The vital layer does not arrest the cercariae, as they were found to pass through a layer formed by the passage for half an hour of aluminated water. The same result followed in another test made by passing newly forming alum precipitate on a small area of sand for an hour thus producing an abnormally thick layer. This, too, offered no obstacle to the leechlike progression of cercariae, as they were found swimming in the filtrate 24 hours later. The layer of sand presents no insuperable barrier, for very active cercariae were found in the filtrate within 1 hour after addition to the inflow of aluminated water, a depth of 75 cm. of sand having been traversed in the interval. Sand of the finest grain used in filtration proved inefficient."

(*g*) *Experience of Egypt.*

The above cited particulars are the most important in the literature. However, it was supposed that the managers of water-purification works in Egypt, where schistosomiasis is widespread, accumulated experience which remains unpublished. In order to acquire these data, one of us (G.W.) went to Egypt where some interesting information could be gathered in Alexandria, Cairo and Tanta. The most important impression was that almost all managers of water-purification works, sanitary officers and laboratory workers proved satisfied that the standard methods of purification of water in use in Egypt are sufficient to remove the danger of infection with schistosomiasis. This opinion was reached through the experience of many years which shows that people who live in towns and avoid drinking unpurified water or bathing in natural basins do not get

infected with schistosomiasis in spite of frequent bathing in tap water (cold showers) and of drinking much unboiled tap-water.

It may be emphasized that the three mentioned towns are applying the same usual processes, i.e. (1) precipitation by aluminium sulphate at the rate of 80/1,000,000 ; (2) filtration for which a standardized quartz sand is used, and (3) chlorination either by a solution of chlorine 0·85/1,000,000 or by chloramine containing 0·4/1,000,000 of available chlorine. The water is supplied to the consumer on an average about 6 hours after it has left the water works.

3.—Experiments of the Present Authors.

In view of the conflicting references and owing to the absence of sufficient scientific proof as to which of the processes used in the purification of water in Egypt is responsible for the disappearance of the cercariae, laboratory experiments were undertaken with the object of finding a good and practical method of eliminating the schistosome cercariae.

(a) *Test Materials used for Experiments.*

The necessary material, i.e., the infected snails shedding out cercariae, were brought from Egypt. They were kept in glass jars in soft tap water in which were placed branches of *Potamogeton lucens* (for food and for aeration) and sometimes pieces of lettuce leaves for food. In the winter time the jars were aerated during the night by an electrical aerator and during the day they were placed on the window in direct sunlight. In the summer direct sunlight was avoided because excessive warming proved fatal to the snails.

Fresh cercariae were used for each experiment. For this purpose single snails were put in test tubes of 9 cm. height and 1½ cm. wide, three-quarters filled with water and placed in the light at 9 or 10 o'clock in the morning. The cercariae began to appear in the water at once ; in the summer time, the maximum output of the cercariae was between 10 a.m. and 1 p.m., in the winter time it was between noon and 2 p.m. Thus the numbers of the cercariae necessary for experiments were usually sufficient at about 2 p.m.

If not disturbed the cercariae sank slowly to the lower layers of the water, but at the slightest agitation of the test tube they quickly rose to the surface to form a dense layer. This layer was taken out by a pipette and served as test material ; 0·5 c.c. of this material contained twenty to fifty cercariae.

The *Bulinus* snails (infected with the cercariae of *S. haematobium*) were very difficult to keep in the laboratory and during the summer months died quickly. However, they were maintained for some 2 months during the winter (*ca* 15° C.) when the experiments could be completed. The *Planorbis* snails (infected with the cercariae of *S. mansoni*) lived well even in summer. In order to make the comparison reliable, only those experiments are quoted in this paper which were made with both species under identical conditions during December-January at an average temperature of 15° C.

The water used for the experiments was taken from the river Yarkon at the point called "Ten Mills" which was found suitable for the intended water-purification plant. The water, either plain or purified, was kept in bottles, sometimes for several days before the experiments. The purification was performed by laboratory methods: fresh river water was clarified by calcium hydroxide and aluminium sulphate and filtered through filter paper.

The snails were collected in localities in Egypt (El-Marg, Maady) reputed for high infection rate with schistosome parasites in both man and snails. We performed the following experiments with *Planorbis* snails in order to ascertain that the cercariae which constituted our test material were really *Schistosoma*. Water containing cercariae was applied to the bare skin on the back of white mice for a period of half an hour. A month later some of the mice proved negative, some had young schistosomes in the liver, while some had developed couples of adult schistosomes in the liver and in the mesenteric veins. (Similar experiments with the *Bulinus* snails were not performed.)

In all the experiments the ingredients were mixed in test tubes of about 10 c.c. volume and immediately poured out into a flat glass dish 3 cm. in diameter in which the liquid remained up to the end of observation. The effect was observed through a binocular microscope. In all the experiments control tubes were set up.

(*b*) *Experiments with Aluminium Sulphate* ($Al_2(SO_4)_3$).

A solution of aluminium sulphate was added separately to the cercariae of both species, suspended in plain Yarkon water, in such a proportion that solutions of 20/1,000,000, 50/1,000,000 and 100/1,000,000 $Al_2(SO_4)_3$ were formed. In the latter two solutions the cercariae were entangled in the flocs formed, and sank with them to the bottom. However, in the course of $\frac{1}{2}$ to 1 hour, they freed themselves and swam above the sediment as vigorously as in the control tubes. In the tubes with the solution of 20/1,000,000 the cercariae of both species swam normally all the time in spite of the flocs. In all the tubes fully active cercariae were found after 24 hours, i.e., as in the control tube.

Conclusion: Aluminium sulphate in the strongest concentration as employed for purification of water does not affect the cercariae.

(*c*) *Experiments with Lime Water and with Alkaline Buffer Solutions.*

Lime, $Ca(OH)_2$, is often used in the course of the purification of water in order to soften it or to accelerate the coagulation of aluminium sulphate. It was, therefore, important to ascertain whether it affects the cercariae.

Two test tubes containing *S. haematobium* cercariae in raw water were filled up to 5 c.c. with a solution of calcium hydroxide calculated so as to neutralize the carbonate hardness in one of the tubes and to make an excess of calcium hydroxide corresponding to 1° of hardness in the other. The pH was 11·1 in the first tube and a little higher in the second. A third tube was filled up with plain water (without calcium hydroxide) as a control. Immediately after the addition of the lime a precipitate was produced and most of the cercariae were entangled in it and sank with it to the bottom. However, after 30 minutes a proportion of them freed themselves and were swimming normally in the

supernatant fluid. This was also the case 1 hour after the beginning of the experiment. Next morning, i.e. *ca.* 20 hours later, there were a few cercariae still swimming in the first tube, all were dead in the second one, while in the control tube the cercariae were normally active.

0·5 c.c. of a suspension of cercariae of *S. mansoni* was put into each of four tubes and to three of them was added, up to the 5·0 c.c. mark, raw water and lime water in such a proportion as to make the excess of calcium hydroxide correspond to 1, 2 and 3 degrees of hardness respectively. To the fourth tube 4·5 c.c. of plain water was added to serve as control. In order to observe the pH an indicator (*Cresol-red* for pH up to 8·8 and *Alizarine-gg* for higher pH) which has been found to be harmless to the cercariae, was added to all the tubes.

After 4 hours all the cercariae remained as active as before, while the pH in the test tubes containing lime water decreased from 11 to 9·8 (the pH in the control tube remained unchanged 8·5). Next morning, i.e. 20 hours after the start of the experiment, all the cercariae in all the test tubes were equally active.

In order to ascertain whether the high pH formed by calcium hydroxide affects the cercariae a similar experiment was made in the following way : Two equal rows of five test tubes each were arranged, each tube containing 0·5 c.c. of *S. mansoni* cercariae suspension and 4·5 c.c. of lime solution in Yarkon water of various strengths. One row was provided with indicator while the other row remained without indicator. At the start, the respective pH were : 11·1, 11·2, 11·3, 11·5, 11·6. In the test tubes with pH 11·6 all the cercariae were dead after 12 minutes (the pH then became reduced to 11·5). In other test tubes and in the control tube the cercariae were unchanged after 2 hours, during which interval the pH in the test tubes became 11·1, 11·2, 11·2, 11·2 and 11·2 respectively.

In order to ascertain whether the lethal effect observed in the latter experiment was caused by the calcium hydroxide itself, or by the high concentration of OH-ions, experiments with buffer solutions were made. In a sodium borate buffer solution (according to SÖRENSEN) containing 1·0 to 1·6 per cent. of total salts, all cercariae were destroyed at pH 11·3 during 70 minutes ; at pH 11·7—in 7 minutes—and at pH 12·1 and above immediately. Similar solutions were then prepared in such a manner that the total salt content was half that of the previous solution. The cercariae were dead after a little longer period, namely at pH 11·4 after 90 minutes, at pH 11·5 in 60 minutes, at pH 11·7 after 35 minutes and at pH 12·0 after 5 minutes.

In order to exclude the possible specific sterilizing effect of boric salts, similar experiments were carried out with phosphate buffer solution, containing about 0·5 per cent. total salts. At pH 11·2 some cercariae were still alive after a period of 2½ hours, at pH 11·4 all were dead after 30 minutes, and at pH 11·9 after 5 minutes. Thus the result proved to be similar to those carried out with the borate buffer.

It may be concluded from these experiments that in the mentioned buffer solutions the high pH was mainly responsible for the death of the cercariae, while the salts themselves exert a comparatively slight effect on the cercariae.

A pH of 11·6 is however never reached when water is treated with $Ca(OH)_2$ in water plants. Thus it is to be expected that $Ca(OH)_2$ treatment as employed in the purification plants will not be effective against schistosome cercariae.

(*d*) *Experiments with Lime Water and Aluminium Sulphate.*

Aluminium sulphate is usually added to the treated water immediately after the addition of the lime. In order to observe the combined effect of these substances on the cercariae the following experiments were performed :—

To three test tubes each containing 1 c.c. of a suspension of cercariae of *S. mansoni* were added respectively 3·50, 3·75 and 3·90 c.c. of a solution of calcium hydroxide in Yarkon river water having 1° of hardness in excess over the neutralization value, and then filled up to 5 c.c. with a solution of aluminium sulphate (1/1,000). Thus, the proportion of aluminium sulphate in the test tubes was respectively 100, 50 and 20 per million. In

addition to this, a control tube in which the solution of aluminium sulphate and another one in which the solution of calcium hydroxide was replaced by plain water, were added to the series. The pH in the first three tubes was respectively 6·5, 8·5 and 9·0, while in the control tube without lime it was 7·0 and in the other one which was without aluminium sulphate it was 9·5. After 3½ hours the cercariae showed normal vitality, and after 20 hours most of them were still normal.

Four pairs of test tubes were set up as follows : 1 c.c. of the suspension of *S. mansoni* cercariae was placed in each tube ; into the first pair a solution of calcium hydroxide calculated to neutralize the carbonate hardness was added ; into the second pair also calcium hydroxide was added but calculated to form an excess of lime corresponding to 1° of hardness ; both these tubes were filled up to 5 c.c. with a solution of aluminium sulphate calculated to form a concentration of 50/1,000,000. The third and fourth pairs served as controls ; the former contained aquarium water instead of calcium hydroxide while the latter contained water instead of both calcium hydroxide and aluminium sulphate solutions. To one tube of each of the four pairs an indicator was added for the estimation of pH. At the beginning the pH was respectively 9·1, 9·4, 8·0 and 9·2.

After 10 minutes all the cercariae in the first two pairs of tubes were entangled in the sunken flocs but still showed very active movements as if attempting to free themselves. After 25 minutes some of the cercariae freed themselves from the sediment and swam about in the supernatant water. The same was observed 1 hour later (when the pH was respectively 8·9, 9·0, 7·9 and 8·5). After 19 hours almost all cercariae in the two first tubes were dead while in the control tubes they were normally active.

A similar experiment was performed with three tubes, with the difference that calcium hydroxide was calculated to make an excess corresponding to 1°, 2° and 3° of hardness. After the addition of aluminium sulphate, the flocs obscured the view and started to sink slowly. The pH was then 11·1, 11·1 and $<$11·1 respectively. 15 minutes later when the flocs sedimented no cercariae were seen in the supernatant water. 20 minutes from the beginning of the experiment the sediment was examined and it was found that all the cercariae were dead, some of them not being attached to the flocs. In both control tubes the cercariae were normally active. The death of the cercariae in the experimental tubes is to be ascribed to the high pH, but it is, however, to be remarked that the presence of flocs which drive the cercariae to the bottom seems to accelerate the lethal action of the alkali. Practically, however, water treated by both chemicals never attains a pH over 9.

From a practical point of view the treatment with lime and aluminium sulphate will not be effective against cercariae.

(*e*) *Experiments with Chlorine.*

The action of the following three forms of chlorine on the cercariae of both species of *Schistosoma* was tested : (*a*) solution of gaseous chlorine in water, (*b*) solution of sodium hypochlorite prepared from eau de Javel, (*c*) solution of chloramine prepared as follows :—

" Chlorine water " containing about 2 grammes of chlorine per litre was prepared by introducing gaseous chlorine into the water. Two grammes of ammonium chloride (NH_4Cl) were dissolved in 100 c.c. of this chlorine water and this solution was left to stand for about 2 hours at room temperature ; 2½ c.c. of this solution dissolved in one litre of water formed the stock chloramine solution used in our experiments and it contained from one to two per million of active chlorine. There always was a difference between the amount of chlorine

actually found by analysis in the stock solution and the amount calculated from the chlorine content of the chlorine water. Only the result of the analysis was taken into account in our experiments. The stock solution contained about 15 mg. NH_3 per litre and the ratio of the available chlorine to NH_3 was about 1 : 7.

All the solutions were made with the river water clarified by calcium hydroxide and aluminium sulphate and filtered through filter paper. The reaction of the water was tested at the beginning of the experiment, and pH varied from 7·5 to 8·9. All experiments were made with 0·5 c.c. of the suspension of cercariae to which firstly water and then the stock solution of either form of chlorine was added. In each experiment a control tube was set up which contained 0·5 c.c. of the suspension of cercariae and 4·5 c.c. of water. In all experiments a parallel row of tubes was set up in which the indicator, O-Tolidin was added to estimate the amount of available chlorine at any moment. The estimation of the amount of chlorine was made colorimetrically by comparison with standard coloured sealed tubes, three times during each experiment : at first the amount of chlorine in the stock solution was determined and the concentration of chlorine at the beginning in the experimental tubes was calculated accordingly; the next test was made 10 minutes and the third one 35 minutes after the addition of the stock solution to the tubes containing cercariae.

The active chlorine was disappearing with varying rapidity not only in different experiments but also in different tubes of the same experiment. It was found that when the stock solution of chlorine was added to plain water with or without cercariae, the amount of available chlorine fell down rapidly in most cases, probably being absorbed by the organic matter dissolved in the water, or by the suspended living organisms. This absorption proceeded at a high rate usually during the first 10 minutes and afterwards the remaining chlorine diminished very slowly.

Concentrations of chlorine, 1/1,000,000, kill the cercariae at once, irrespective of the form of chlorine. Low concentrations act slowly and the cercariae pass through several stages of progressive loss of activity up to the absolute and irretrievable loss of motion and transparency which is a sign of death. In order to determine the degree of the effect of chlorine on the cercariae the following seven phases were differentiated :—

0—Normal movements.

1—Irregular and weak movements ; the beginning of sinking.

2—The cercariae are creeping at the bottom or they partly swim above the bottom.

3—All cercariae lie at the bottom and their tails show convulsive lateral motion.

4—The cercariae lie at the bottom, their bodies and tails moving slowly.

5—Some of the cercariae are already dead.

6—All the cercariae are dead.

The results of the experiments are shown on Tables I to VI. The analysis

TABLE I.

EXPERIMENTS ON THE EFFECT OF GASEOUS CHLORINE ON THE CERCARIAE OF *Schistosoma haematobium*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	7·5	0·10	0	0	—	1.20	—	—	—	2.00
2	7·8	0·10	T	0	24	34	2.00	—	—	2.00
3	8·8	0·10	T	0	24	—	—	—	—	2.00
4	7·5	0·20	0·10	0	12	28	—	—	—	2.00
5	8·8	0·20	0·10	0·10	—	21	—	—	—	2.00
6	7·8	0·20	0·12	0·10	15	28	1.04	—	—	2.00
7	7·8	0·20	0·15	0	—	15	—	—	—	1.20
8	7·5	0·30	0·15	0·10	10	17	48	1.10	—	2.00
9	7·8	0·30	0·15	0·15	—	8	45	1.35	—	2.00
10	8·8	0·30	0·20	0·17	12	19	55	—	—	2.00
11	7·8	0·40	0·20	0·20	8	10	31	48	1.12	
12	7·5	0·40	0·25	0·20	5	14	24	43	53	
13	7·5	0·50	0·28	0·25	3	12	29	35	40	
14	7·8	0·50	0·30	0·20	—	7	17	40	58	
15	7·8	0·60	0·30	0·30	—	—	6	28	55	
16	7·5	0·60	0·35	0·30	—	9	—	21	30	
17	7·8	0·80	0·40	0·40	—	7	—	10	24	
18	7·8	0·60	0·40	0·28	5	—	15	35	55	
19	8·8	0·60	0·40	0·40	—	6	21	40	48	
20	7·5	0·80	0·45	0·40	—	—	7	—	27	
21	7·8	1·00	0·60	0·45	—	5	—	10	20	
22	7·8	1·20	0·60	0·50	—	4	—	8	18	
23	8·8	1·00	0·70	0·70	8	—	—	20	30	

TABLE II.

EXPERIMENTS ON THE EFFECT OF GASEOUS CHLORINE ON THE CERCARIAE OF *Schistosoma mansoni*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·0	0·18	0·10	0·10	23	31	—	—	—	1.20
2	8·8	0·18	0·13	0·13	—	19	1.35	—	—	1.35
3	8·0	0·27	0·17	0·17	—	18	56	1.15	—	1.17
4	8·8	0·27	0·25	0·23	9	18	45	1.05	1.32	
5	8·0	0·36	0·25	0·25	—	10	18	52	1.15	
6	8·8	0·36	0·30	0·30	—	—	12	36	47	
7	8·0	0·45	0·30	0·30	—	8	18	23	55	
8	8·8	0·45	0·35	0·33	5	8	12	20	32	
9	8·0	0·54	0·40	0·35	—	5	—	22	24	
10	8·8	0·54	0·42	0·40	2	4	10	15	24	
11	8·0	0·70			—	3	—	10	18	
12	8·8	1·72	0·60	0·60	—	—	7	10	11	
13	8·8	1·08	0·70	0·70	—	5	—	4	5	
14	8·0	1·10	0·85	0·75	—	—	—	—	5	

* Remarks: The experiments are arranged according to the amount of available chlorine as estimated 10 minutes after the addition of the stock solution.
Time is shown in minutes.
" T " means traces of chlorine.
Amount of chlorine is given per million.
pH relates to the water used for the experiment.
The meaning of the grades of the effect is given on page 557.
The underlined figures denote the cases of discrepancies in correlation between the amount of available chlorine employed and the results of the experiment.

TABLE III.

EXPERIMENTS ON THE EFFECT OF CHLORAMINE ON THE CERCARIAE OF *Schistosoma haematobium*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·2	0·045	T	0	18	—	—	—	—	2.00
2	8·1	0·075	0·05	T	6	18	—	—	—	1.15
3	8·2	0·07	T	0	—	15	—	—	—	2.00
4	8·2	0·09	0·05	0·05	12	18	—	—	—	2.00
5	8·2	0·10	0·05	0·05	—	9	1.27	—	—	2.00
6	8·2	0·12	0·10	0·01	—	7	13	42	—	2.00
7	8·1	0·12	0·10	0·10	5	8	19	1.09	—	1.15
8	8·2	0·14	0·12	0	—	11	—	—	—	1.00
9	8·2	0·13	0·13	0·10	—	7	—	1.32	—	2.00
10	8·1	0·12	0·12	0·10	3	30	—	—	—	1.15
11	8·2	0·15	0·13	0·13	—	5	14	47	—	2.00
12	8·1	0·20	0·18	0·15	—	3	5	21	58	
13	8·2	0·20	0·16	0·13	—	—	6	15	30	
14	8·1	0·20	0·20	0·18	3	5	11	20	41	
15	8·2	0·27	0·20	0·20	4	10	15	35	—	1.00
16	8·1	0·30	0·27	0·22	—	—	4	7	10	
17	8·1	0·30	0·28	0·28	—	—	3	10	18	
18	8·2	0·36	0·30	0·28	—	—	3	8	10	
19	8·2	0·42	0·30	0·25	—	4	7	13	18	
20	8·2	0·45	0·25	0·28	—	—	—	—	5	
21	8·2	0·54	0·40	0·35	—	—	5	7	11	
22	8·2	0·70	0·50	0·47	—	—	3	—	8	
23	8·2	0·55	0·80	0·55	—	—	—	—	6	
24	8·2	1·10	0·75	0·60	—	—	—	—	3	
25	8·2	1·30	0·90	0·70	—	—	—	—	1	

TABLE IV.

EXPERIMENTS ON THE EFFECT OF CHLORAMINE ON THE CERCARIAE OF *Schistosoma mansoni*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·1	0·06	T	T	2.00	—	—	—	—	2.00
2	8·2	0·25	0·10	0·10	10	—	24	—	—	1.30
3		0·12	0·12	0·10	—	5	19	1.00	—	1.30
4	8·1	0·17	0·12	0·10	—	21	19	2.00	—	2.00
5	8·2	0·27	0·13	0·12	5	7	29	—	—	1.30
6	8·1	0·20	0·15	0·13	—	13	26	1.00	2.00	—
7	8·2	0·32	0·15	0·15	—	5	18	—	—	1.30
8	8·2	0·36	0·18	0·17	—	3	11	32	1.10	
9	8·1	0·26	0·20	0·15	8	—	15	18	43	
10	8·2	0·45	0·20	0·18	—	2	7	18	30	
11		0·20	0·20	0·20	—	3	8	25	34	
12	8·1	0·31	0·20	0·20	3	4	11	14	45	
13	8·2	0·49	0·20	0·20	—	2	6	10	25	
14	8·2	0·54	0·22	0·22	—	2	—	9	20	
15	8·1	0·39	0·24	0·23	—	2	7	10	12	
16		0·30	0·28	0·25	—	—	—	6	10	
17	8·1	0·52	0·30	0·25	—	3	4	—	7	
18	8·1	0·78	0·32	0·38	—	—	—	—	4	
			0·50							

* Remarks : The experiments are arranged according to the amount of available chlorine as estimated 10 minutes after the addition of the stock solution.

Time is shown in minutes.

" T " means traces of chlorine.

Amount of chlorine is given per million.

pH relates to the water used for the experiment.

The meaning of the grades of the effect is given on page 557.

The underlined figures denote the cases of discrepancies in correlation between the amount of available chlorine employed and the results of the experiment.

TABLE III.

EXPERIMENTS ON THE EFFECT OF CHLORAMINE ON THE CERCARIAE OF *Schistosoma haematobium*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·2	0·045	T	0	18	—	—	—	—	2.00
2	8·1	0·075	0·05	T	6	18	—	—	—	1.15
3	8·2	0·07	T	0	—	15	—	—	—	2.00
4	8·2	0·09	0·05	0·05	12	18	—	—	—	2.00
5	8·2	0·10	0·05	0·05	—	9	1.27	—	—	2.00
6	8·2	0·12	0·10	0·01	—	7	13	42	—	2.00
7	8·1	0·12	0·10	0·10	5	8	19	1.09	—	1.15
8	8·2	0·14	0·12	0	—	11	—	—	—	1.00
9	8·2	0·13	0·13	0·10	—	7	—	1.32	—	2.00
10	8·1	0·12	0·12	0·10	3	30	—	—	—	1.15
11	8·2	0·15	0·13	0·13	—	5	14	47	—	2.00
12	8·1	0·20	0·18	0·15	—	3	5	21	58	
13	8·2	0·20	0·16	0·13	—	—	6	15	30	
14	8·1	0·20	0·20	0·18	3	5	11	20	41	
15	8·2	0·27	0·20	0·20	4	10	15	35	—	1.00
16	8·1	0·30	0·27	0·22	—	—	4	7	10	
17	8·1	0·30	0·28	0·28	—	—	3	10	18	
18	8·2	0·36	0·30	0·28	—	—	3	8	10	
19	8·2	0·42	0·30	0·25	—	4	7	13	18	
20	8·2	0·45	0·25	0·28	—	—	—	—	5	
21	8·2	0·54	0·40	0·35	—	—	5	7	11	
22	8·2	0·70	0·50	0·47	—	—	3	—	8	
23	8·2	0·55	0·80	0·55	—	—	—	—	6	
24	8·2	1·10	0·75	0·60	—	—	—	—	3	
25	8·2	1·30	0·90	0·70	—	—	—	—	1	

TABLE IV.

EXPERIMENTS ON THE EFFECT OF CHLORAMINE ON THE CERCARIAE OF *Schistosoma mansoni*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·1	0·06	T	T	2.00	—	—	—	—	2.00
2	8·2	0·25	0·10	0·10	10	—	24	—	—	1.30
3		0·12	0·12	0·10	—	5	19	1.00	—	1.30
4	8·1	0·17	0·12	0·10	—	21	19	2.00	—	2.00
5	8·2	0·27	0·13	0·12	5	7	29	—	—	1.30
6	8·1	0·20	0·15	0·13	—	13	26	1.00	2.00	—
7	8·2	0·32	0·15	0·15	—	5	18	—	—	1.30
8	8·2	0·36	0·18	0·17	—	3	11	32	1.10	
9	8·1	0·26	0·20	0·15	8	—	15	18	43	
10	8·2	0·45	0·20	0·18	—	2	7	18	30	
11		0·20	0·20	0·20	—	3	8	25	34	
12	8·1	0·31	0·20	0·20	3	4	11	14	45	
13	8·2	0·49	0·22	0·20	—	2	6	10	25	
14	8·2	0·54	0·24	0·22	—	2	—	9	20	
15	8·1	0·39	0·28	0·23	—	2	7	10	12	
16		0·30	0·30	0·25	—	—	—	6	10	
17	8·1	0·52	0·32	0·25	—	3	4	—	7	
18	8·1	0·78	0·50	0·38	—	—	—	—	4	

* Remarks : The experiments are arranged according to the amount of available chlorine as estimated 10 minutes after the addition of the stock solution.
Time is shown in minutes.
" T " means traces of chlorine.
Amount of chlorine is given per million.
pH relates to the water used for the experiment.
The meaning of the grades of the effect is given on page 557.
The underlined figures denote the cases of discrepancies in correlation between the amount of available chlorine employed and the results of the experiment.

TABLE V.

EXPERIMENTS ON THE EFFECT OF SODIUM HYPOCHLORITE ON THE CERCARIAE OF *Schistosoma haematobium*.*

No.	pH.	Amount of available chlorine estimated at			Time at which various phases of the effect began.					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1		0·20	0·10	0·10	—	20	50	—	—	2.00
2		0·40	0·17	0·13	12	20	30	1.12	—	2.00
3	8·9	0·20	0·17	0·17	12	20	30	40	—	1.30
4		0·60	0·23	0·18	—	18	32	60	1.12	
5		1·00		0·18	—	15	25	32	1.20	
6		0·40	0·27	0·23	10	—	16	25	1.40	
7		0·80	0·28	0·20	—	—	15	28	42	
8		0·90	0·29	0·20	—	—	15	27	45	
9		0·60	0·30	0·25	—	—	10	12	34	
10		0·80	0·33	0·25	—	—	10	17	27	
11	8·9	0·40	0·35	0·35	—	14	—	18	40	
12		1·00	0·42	0·25	—	—	12	18	28	
13		1·20	0·48	0·25	—	—	—	14	26	
14	8·9	0·60	0·50	0·45	—	—	—	10	18	
15		1·40	0·55	0·27	—	—	15	20	25	
16	8·9	0·80	0·80	0·40	—	—	—	—	10	
17	8·9	1·00	0·60	0·40	—	—	—	—	10	
18	8·9	1·20	1·00	0·60	—	—	—	8	18	

TABLE VI.

EXPERIMENTS ON THE EFFECT OF SODIUM HYPOCHLORITE ON THE CERCARIAE OF *Schistosoma mansoni.**

No.	pH.	Amount of available chlorine estimated after			Time at which various phases of the effect began					Duration of the observation.
		0	10′	35′	2	3	4	5	6	
1	8·8	0·10	0·05	T	—	—	—	—	—	1.45
2	8·4	0·18	0·10	0·08	29	33	1.37	—	—	1.37
3	8·6	0·20	0·20		—	40	1.18	—	—	1.30
4	8·4	0·27	0·20	0·15	7	25	1.20	—	—	1·33
5	8·4	0·36	0·24	0·22	8	21	26	38	1.04	
6	8·6	0·30	0·25		—	21	26	34	1.09	
7	8·8	0·30	0·25	0·18	15	27	1.24	—	—	1.45
8	8·6	0·40	0·28		—	9	17	23	29	
9	8·8	0·40	0·30	0·28	4	15	45	50	1.05	
10	8·4	0·45	0·30	0·30	—	8	15	29	35	
11	8·4	0·54	0·35	0·35	4	6	—	11	24	
12	8·8	0·50	0·38	0·38	2	3	12	29	36	
13	8·6	0·50	0·40		—	6	—	13	25	
14	8·8	0·60	0·42	0·40	2	8	13	18	21	
15	8·4	0·72	0·45	0·45	—	4	7	10	16	
16	8·6	0·60	0·50		—	4	9	13	19	
17	8·4	1·08	0·60	0·58	—	—	4	7	15	
18	8·8	0·80	0·63	0·63	2	5	9	14	19	
19	8·6	0·80	0·70		—	3	5	—	12	
20	8·6	1·00	0·80		—	1	3	7	12	
21	8·8	1·00	0·80	0·73	—	—	6	10	13	

* Remarks : The experiments are arranged according to the amount of available chlorine as estimated 10 minutes after the addition of the stock solution.

Time is shown in minutes.

" T " means traces of chlorine.

Amount of chlorine is given per million.

pH relates to the water used for the experiment.

The meaning of the grades of the effect is given on page 557.

The underlined figures denote the cases of discrepancies in correlation between the amount of available chlorine employed and the results of the experiment.

of these tables shows that there is no absolute correlation between the amount of chlorine and the lethal effect. If we arrange the experiments according to the initial amount of chlorine, calculated from the stock solution, the corresponding row of figures marking the death time will show a gradual fall, but with a number of discrepancies (see Table VIII, left row, and also Tables I to VI, the rows of grade 6 of the effect). It will appear that in some experiments a smaller initial amount of chlorine kills the cercariae quicker than a larger amount in one of the next experiments. However, if the experiments are arranged according to the amount of chlorine determined 10 minutes after the addition of the stock solution of chlorine, the number of discrepancies will be smaller (see Table VIII, right row).

It is hence concluded that the amount of chlorine which remains 10 minutes after the addition of the stock solution is a better criterion for forecasting the effect than the calculated initial amount. Therefore, in Tables I to VI the experiments are arranged according to the results of the estimation of the chlorine after 10 minutes.

It is difficult to explain the discrepancies in the gradual rise of the lethal effect of chlorine. They may possibly be due to different content of organic substances, such as the excreta of the snails or of the cercariae, in various samples of cercariae material. These substances certainly absorb a part of the available chlorine and their amount could not be estimated. We were not able to avoid them for we did not know how to separate the cercariae from the water into which they were discharged by the snail, without injuring them.

In any case, it is evident from Tables I to VI that the amount of chlorine, and particularly that amount which remains after 10 minutes contact with the water containing cercariae, is responsible for the rapidity of the lethal effect. On the other hand, each of the three forms of chlorine used exerts different action on each species of the parasite. Thus, six figures may be established which denote the minimum initial concentration of available chlorine which is certainly lethal for the cercariae after a more or less prolonged period (see Table VII, first row). However, in practice it is desirable to have the lethal effect assured within a limited time, say 30 minutes necessary for the water to pass from the purification plant to the consumer. In this case another six figures should be chosen (see Table VII, second row). Other figures (see Table VII, third and fourth rows) are to be taken in cases when the amount of available chlorine *after* 10 minutes is taken as the criterion.

It is evident from Table VII that chloramine is at least twice as powerful as both gaseous chlorine or sodium hypochlorite. The minimum initial concentration which has lethal effect in respect to both species of schistosome cercariae is 0·45 per million as compared with 0·8 and 1·0 per million for gaseous chlorine and sodium hypochlorite respectively. If the after-10-minutes estimation of available chlorine has to be taken as a criterion, 0·22 per million has to be compared with 0·6 and 0·42 respectively.

TABLE VII.

MINIMUM CONCENTRATIONS OF ACTIVE CHLORINE (PER MILLION) SHOWING LETHAL EFFECT ON SCHISTOSOME CERCARIAE.

Form of chlorine.	Species of *Schistosoma* cercariae.	According to preliminary estimation of Cl.		According to the estimation after 10 min.	
		m.l.c.	m.p.c.	m.l.c.	m.p.c.
Gaseous chlorine	*haematobium*	0·40	0·80	0·25	0·60
	mansoni	0·27	0·54	0·25	0·40
Chloramine	*haematobium*	0·30	0·30	0·22	0·22
	mansoni	0·36	0·45	0·18	0·22
Natr. hyperchlorid	*haematobium*	0·40	1·00	0·23	0·42
	mansoni	0·36	0·50	0.28	0·40

m.l.c. = minimum lethal concentration.
m.p.c. = minimum practical concentration (lethal within the limits of 30 minutes).

TABLE VIII.

NUMBER OF DISCREPANCIES IN CORRELATION BETWEEN THE AMOUNT OF AVAILABLE CHLORINE EMPLOYED AND THE RESULTS OBTAINED.

Form of chlorine.	Species of *Schistosoma* cercariae.	If ordered according to the preliminary estimation of Cl.	If ordered according to the estimation after 10 minutes.
Gaseous chlorine	*haematobium*	6	5
	mansoni	1	1
Chloramine	*haematobium*	5	6
	mansoni	9	2
Natr. hyperchlorid	*haematobium*	9	7
	mansoni	7	5
Total		37	26

It can also be seen from Table VII that cercariae of *S. haematobium* are more sensitive towards chloramine and more resistant towards gaseous chlorine and sodium hypochlorite, while it is the reverse with the cercariae of *S. mansoni.* The obvious conclusions may be drawn that when the action of the chlorine is required against both species of *Schistosoma*, concentrations of the chlorine should be applied which are lethal for the more resistant one. These concentrations are underlined in Table VII.

It has to be emphasized that the environmental conditions in our experiments were more advantageous for the cercariae than they are in the purification plant. As already mentioned above, the water containing cercariae, as used in the experiments, was inevitably polluted by organic substances excreted by the snail or by the cercariae themselves and therefore the absorption of available chlorine proceeded undoubtedly more rapidly than it might do in a plant, where water clarified by aluminium sulphate and filtered is subjected to chlorination. Further, the experiments took place in small open glass dishes with comparatively large surface and hence the aeration of the water was better than it is in big tanks in a plant. Thus, it may be supposed that in the plant practice the minimum concentrations of available chlorine which are necessary to kill the cercariae are smaller than those indicated on Table VIII.

(*f*) *Irreversibility of the Effect of Chlorination.*

In order to determine whether the effect of the chlorination upon the cercariae may be stopped or reversed by the elimination of chlorine the following experiments were performed.

Cercariae of *S. mansoni* were subjected to the action of sodium hypochlorite with the initial amount of available chlorine of 0·6 per million. After 25 minutes, when the cercariae were lying on the bottom of the test tube in the 2nd phase, the supernatant water was poured out and replaced by plain water. No recovery of the cercariae could be noticed and they began to die after 1 hour 20 minutes. To test tubes arranged in pairs and containing *S. mansoni* cercariae sodium hypochlorite was added to make the concentration of available chlorine respectively 0·60, 0·66, 0·80 and 0·88 per million. In one test tube out of every pair the experiment proceeded normally to the end. In the second one the solution was decanted after 10 minutes, when the cercariae started to sink, and was replaced by plain water. On comparing the effect in both rows only insignificant difference or no difference at all could be noticed. For instance, the effect in the decanted test tubes containing 0·66 per million available chlorine was somewhat weaker than in the non-decanted one, though the injurious action of chlorine continued after the decantation. In the decanted test tube containing 0·88 per million chlorine all the cercariae were destroyed during the same period of time while in the non-decanted test tube only a part of the cercariae died.

Similar experiments were carried out with chloramine—0·66 and 0·88 available chlorine per million—and with gaseous chlorine—0·22 and 0·35 per million. The decantation of the solution of chloramine after 5 and 7 minutes exposure respectively did not change the results, in both pairs of tubes the cercariae being dead after 10 and 17 minutes respectively. In the experiments with the gaseous chlorine *most* of the cercariae were killed in the decanted tubes after 2 hours while in the non-decanted control *all* cercariae were dead within that period.

It may be concluded from the above experiments that the contact with the chlorine (irrespective of the three forms used) during a certain period exerts on the cercariae (*S. mansoni*) an adverse effect which cannot be stopped in its further development or be reversed by the removal of the cercariae from the injurious solution. Consequently it may be supposed that in order to destroy the cercariae it is sufficient to expose them to the effect of a solution of chlorine for a certain minimum period (depending on the concentration of available chlorine) which does not necessarily make the effect demonstrable. In other words, the minimum lethal concentrations given on Table VII may prove excessive in plant practice.

(g) *Experiments with Filtration.*

In order to study the possibility of retaining the cercariae by filtration, an experimental filter was constructed. It consisted of three iron pipes 10, 15 and 50 cm. long respectively and 6 cm. in diameter. Various combinations of these pipes made a filter of varying height, from 10 to 75 cm., which was filled up with sand of specified size, generally used for the filtration of water. The filter was closed below by a copper net to keep the sand in position and by a collecting funnel. To the upper end of the filter a double-walled funnel was adjusted, its outer wall being higher than the inner one. The water entered the filter through a pipe between these walls and overflowed the inner wall without disturbing the surface of the sand. The inflow was regulated by a cock and during the filtration the surface of the water was constantly kept 2 to 3 cm. above the sand.

Several experiments were made with the cercariae of both *S. mansoni* and *S. haematobium*. Plain water containing them was filtered through a freshly set up, clean filter filled up with water. When the height of the sand was 10 cm. the cercariae appeared in the first amount of filtrate though only part of them passed eventually. When 50 and 75 cm. height was used, they appeared after some delay and in still smaller proportion.

In order to test the so-called " biological layer " which is formed on the surface of the sand filter in the course of filtration and which is essential for the filtration of bacteria the following method was used : A layer was formed on the surface of a 10 cm. high sand column by passing through it 8 litres of water immediately after treating it with calcium hydroxide (calculated to neutralize 90 per cent. of the carbonate hardness) and with aluminium sulphate (100/1,000,000). It took almost half an hour for all this water to pass and the flocs formed on the sand a layer of such a density that the water passed in drops. Thereafter fresh cercariae of *S. haematobium* were put in the filtering water and a few of them could be recovered in the first 100 c.c. of the filtrate. This experiment cannot be regarded as conclusive for the retaining power of the " biological layer " may differ from

the aluminium hydroxide layer. Nevertheless it shows the behaviour of the cercariae in the filter and suggests that the actual " biological layer " may also be successfully passed by the cercariae.

Conclusion : A 75 cm. high layer of specified sand or a layer of aluminium hydroxide flocs cannot retain all the schistosome cercariae.

(*h*) *Experiments with High Pressure.*

There exists a patent for sterilization of various materials by high pressure. In order to study the suitability of this method for destruction of the schistosome cercariae the following experiments were carried out.

Small test tubes filled with water containing the cercariae were covered by a layer of neutral paraffin oil and a thin rubber membrane to permit the transmission of the pressure to the interior of the tubes. The tubes were then put into water in a special apparatus developing high pressure. Four experiments made with various pressures gave negative results. In one of the experiments the cercariae remained for 6 hours under a pressure of 200 atmospheres and yet the vitality of the cercariae remained equal to that of the cercariae in the control tube which was similarly closed but remained under normal pressure.

4.—Conclusions.

The existing data on the destruction of the cercariae of *Schistosoma* during the purification of water, as quoted in the literature, are insufficient and contradictory.

The long empirical experience of the Egyptian water purification plants suggests that the standard methods used for the purification of water rids it of cercariae.

The usual clarification of water by means of aluminium sulphate alone or with the preliminary treatment with lime, even when these substances are employed in maximum quantities generally in use, does not affect the cercariae of *Schistosoma*.

High pressure, up to 200 atmospheres during 6 hours does not injure them.

The standard filtration of water through sand is ineffective.

High alkaline reaction of pH 11·5 to 11·6 kills the cercariae. The presence of $Al(OH)_3$ flocs which drive the cercariae to the bottom seems to increase the effect of the alkali, *i.e.*, a lethal effect is already produced at pH 11·1. However, such a high alkalinity cannot be attained in the water treatment plant, and therefore this interesting fact has no practical value.

The cercariae of *Schistosoma* are killed by chlorine. The time necessary for a sure action of this factor depends on the quantity of active chlorine in the

treated water and on the form in which it is used, *viz.* (*a*) gaseous chlorine, (*b*) sodium hypochlorite and (*c*) chloramine.

(1) *Gaseous chlorine* dissolved in the water, kills the cercariae with certainty in $1\frac{1}{4}$ hours when the *initial* concentration of available chlorine is 0·40 or more per million. Smaller amounts do not give a sure action even during a much prolonged period. In cases when the contact between the chlorine and the cercariae is to be limited to half an hour, a lethal effect can be produced by an initial concentration of not less than 0·8 parts of chlorine per million.

(2) *Sodium hypochlorite* gives a sure effect in an *initial* concentration of 0·4 per million of available chlorine after at most 1 hour 40 minutes, weaker concentrations being uncertain even after longer periods. If the action is to be limited to 30 minutes an initial concentration of 1·0 per million is necessary.

(3) *Chloramine*, as prepared according to the formula described above, kills the cercariae with certainty in 1 hour 10 minutes when the *initial* concentration of available chlorine is 0·36 per million. If the 30 minutes limit is required 0·45 per million of available chlorine should be applied.

Thus, chloramine is the most effective form of chlorine tested.

The absorption of available chlorine by the treated water is an important factor for the calculation of the solutions. If the initial amount of the available chlorine was not large enough, the remaining quantity proves, after the initial absorption, insufficient to exert the necessary action. In cases when the treated water contains comparatively little dissolved (or suspended) material, the absorption of the chlorine is slight, in which case a comparatively small initial amount of available chlorine suffices to kill all the cercariae. The amount of the available chlorine *which remains in the water 10 minutes after the addition of the stock solution* indicates the value of the concentration more exactly than the initial estimation. Therefore, in order to use the chlorine economically it is useful to calculate the solutions on the basis of the amount of available chlorine which remains after 10 minutes contact with the treated water.

If *gaseous chlorine* is used, a sure lethal effect will be obtained if the amount of the available chlorine after 10 minutes will be 0·25 per million, though the action may be concluded after $1\frac{1}{2}$ hours. For a period of exposure limited to 30 minutes, 0·6 per million of available chlorine should be present in the water after 10 minutes.

If *sodium hypochlorite* is used, a sure effect is to be expected during 1 hour 5 minutes if there remains 0·28 per million of available chlorine, and at most during 30 minutes if 0·42 per million or more of available chlorine remains after 10 minutes.

If *chloramine* is used, a lethal effect is certain in less than 30 minutes if 0·22 per million of available chlorine remains after 10 minutes. Lower concentrations are not certain even if longer periods are allowed.

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CORRIGENDUM.

Vol. XXXI, No. 4. January, 1938, p. 471.

Col. CLAYTON LANE: Correspondence on Bancroftian filariasis and the reticulo-endothelial system. 3rd paragraph, line 5, for " What is not known " read " What is now known."

CORRESPONDENCE.

" SELLAR FEVER."

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

May I first be permitted to congratulate Colonel JACK* on a very painstaking and accurate description of a short fever that occurs in the Delhi-Meerut area. I have watched the fever he describes, in Delhi and Meerut from December, 1936, to December, 1937. It had previously appeared in this area in the 1932-33 period and has been absent from the area, in its full clinical form, in the interval. Unlike Colonel JACK, I observed the disease mainly in European soldiers. I have, however, seen the same illness amongst my Indian servants and in a consultant capacity in patients in Indian military hospitals in this area. Needless to say the observation of the rash is handicapped in dark-skinned patients, especially in the very numerous ambulant cases.

In the cause of accuracy and to avoid endless duplication of diagnoses may it be represented that " sella " is saddle and that " saddle back fever " is another name for dengue. The illness seen at Meerut, which varies in no particular from Colonel JACK's description of the illness in these TRANSACTIONS, runs the same clinical course as dengue observed in Cairo in the pandemic, 1927-28. My observations on dengue in Calcutta during 1933-34 resulted in my publishing a short article on " Dengue Temperature Charts," which appeared in the *Journal of the Royal Army Medical Corps*, February, 1936. Only six charts are shown each representing the most typical case observed in the sub-group showing that form of febrile response.

It is not my custom to cite text-books as consolidated descriptions of a disease ill suit the disease as observed under particular circumstances. But *Tropical Medicine* (ROGERS and MEGAW, 2nd Edition, 1935, page 165) gives a map of the distribution of dengue in India. The Sialkot area is marked as a place where dengue occurs. *The Principles and Practice of Medicine* (OSLER and MCCRAE, 11th Edition, 1930, page 354) describes the mouth symptoms, congestion of the pharynx and enlargement of the lymph-glands of the neck. OSLER follows the original description of the disease by Dr. BENJAMIN RUSH in the epidemic in Philadelphia in 1780.

It is my opinion, without proof other than that the maximum incidence of culicine mosquitoes and the maximum incidence of the fever occur at the same

* JACK, W. A. M. (1937). Sellar fever. *Trans. R. Soc. trop. Med. Hyg.*, 31 (3), 281.

time, that *Culex fatigans* is the vector of the illness in the Delhi-Meerut area. Those who follow the Drum travel many miles, and a journey of a thousand miles in 2 days is common in India. The arrival of persons from a distance during the incubation period will explain the occurrence of the illness at Razmak if the culicine mosquito is not to be found there. Hibernating mosquitoes may wake up to feed in a warm closed up hut, even in winter with snow on the ground outside.

I am, etc.,

F. J. O'MEARA.
Major R.A.M.C.

British Military Hospital,
Meerut, U.P., India.
12*th January*, 1938.

ONYALAI.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

DEAR SIR,

With reference to the communication " Onyalai : a Review," by W. K. BLACKIE*, may I contribute a few very belated remarks.

My practice for the last 14 years has been confined almost entirely to natives of the Nyamwezi tribe, with an average of about four thousand new out-patients per year, and about three hundred in-patients. During my residence here, I have made a study of their language, and can claim at least a good working knowledge of Kinyamwezi. Unyamwezi is the name applied to an area of about 60,000 square miles, situated in the Western Province of Tanganyika Territory, with Tabora as its centre.

Dr. BLACKIE is right in advising care and discretion in the interpretation of the natives' names of obscure diseases. The word *kafindo* is the diminutive of *lufindo* which means anus. The duplicated form *kafindofindo* is the name applied to any acute inflammatory condition of the tonsillar or pharyngeal regions, and so covers such different conditions as peritonsillar abscess, ulcerative tonsillitis or severe pharyngitis.

My experience of a condition resembling onyalai is confined to two cases. The first was a girl of about 10 years of age, seen in April, 1936. The parents gave a history of illness of 2 or 3 days only. She was admitted, moribund, with blood oozing from the mouth and nose ; there were haemorrhagic bullae inside the cheeks and under the mucous membrane of the hard and soft palate.

*BLACKIE, W. K. (1937), *Trans. R. Soc. trop. & Hyg.* 31 (2), 207.

Apart from the haemorrhage, the most striking feature was an intense jaundice. She died a few hours after admission.

The second, and probably more typical case was seen in October, 1937, a few days before I read Dr. BLACKIE's review. She was a woman of about 40, and attended as an out-patient complaining of a bloody diarrhoea of 1 day's duration. It so happened that at that time we were experiencing a small epidemic of what was probably bacillary dysentery, and she was treated in the routine way with TOMB's essential oil mixture, a treatment which clears up the majority of these cases of bloody diarrhoea. The next morning she came with her husband, who stated that the diarrhoea had stopped, but that she was too weak to go home; and she was, therefore, admitted. Further examination revealed that she was extraordinarily dull and listless and with haemorrhagic bullae varying from 5 to 10 millimetres in diameter. Shortly after admission she complained of haemorrhage per vaginam; examination showed dark and offensive blood steadily oozing from the vagina. She was not pregnant but she was emphatic that the flow was not menstrual. It is regrettable that no visual examination was made of the vagina and cervix to ascertain exactly the source of the haemorrhage. Bleeding then started from the mouth and nose, and the patient's condition became desperate. Calcium lactate had no effect, and for lack of any other line of treatment I injected into the buttock 10 c.c. of whole blood taken from her husband and injected immediately. I was interested to find, a few days later, when reading Dr. BLACKIE's review, that I had stumbled on the most effective treatment for the condition. Unfortunately the patient's relatives carried her off in the night, and she was not seen again; but it was reported that she died at her village the following day. From the day of her admission until she died the flow per vaginam was continuous, but there was no return of the bloody diarrhoea, and no haematuria. The course of the disease was 5 days, and during the 3 days she was in hospital, the temperature was subnormal.

After reading Dr. BLACKIE's review I made enquiries among the people, and found that this disease was not entirely unknown to some of them, and was dreaded as fatal; but it was so rare that they had no name for it in Kinyamwezi. So striking a condition could hardly have been missed very often during 14 years' native practice.

The two points I would emphasize are (1) that the term *kafindofindo* in Kinyamwezi is not applied to onyalai; and (2) that onyalai must be very rare in Unyamwezi.

I am, etc.,

A. J. KEEVILL.

Moravian Mission Hospital,
Sikonge, Tabora,
Tanganyika Territory.
10th February, 1938.

THE LATE SIR THOMAS STANTON.

We regret to announce that Sir THOMAS STANTON, Chief Medical Adviser to the Secretary of State for the Colonies, a member of the Council, a former Vice-President and a Trustee of the Society, died in London on 25th January last, after a short illness, at the age of 62. The Council, at their meeting on 17th February, 1938, decided to record their appreciation of the services rendered to tropical medicine by Sir THOMAS STANTON, and their sense of loss at his death, in the following resolution :—

> " The President and Council take this opportunity of expressing their profound regret at the death of their esteemed colleague, Sir THOMAS STANTON, one of the founders of the Society and one of its most active supporters.
>
> Sir THOMAS STANTON, by his scientific researches in tropical diseases, rendered eminent service in the advancement of public health in the Tropics, and by his personality endeared himself to all who came in contact with him."

AMBROSE THOMAS STANTON was born in Canada on 14th November, 1875, and was educated at Trinity College, Toronto, where he qualified M.D. in 1899. He spent several years in hospital work before coming to England to take the Conjoint qualification in 1905, and subsequently proceeded to the School of Tropical Medicine at the Royal Albert Dock, where he first met Sir PATRICK MANSON. STANTON'S admiration of MANSON was such that he decided to follow in the footsteps of the master and devote his life to the study of tropical medicine. He was selected to be a Demonstrator at the School, which post he occupied for 2 years, and in 1907, his Chief recommended him to the Colonial Office for appointment as an Assistant in the Institute of Medical Research, Kuala Lumpur.

Here STANTON worked with the late H. FRASER, the Director of the Institute; together they carried out their remarkable studies on the etiology of beri-beri, and proved the disease to be due to a deficiency in diet—one of the early definite proofs that dietetic deficiency is a cause of disease. STANTON was also a keen entomologist, and his work on mosquitoes is considered by some to be as valuable as his work on beri-beri. His name is also associated with a fatal disease which appeared in 1917 among the labourers on rubber estates, and in conjunction with WILLIAM FLETCHER, STANTON showed that the disease, which they subsequently named melioidosis, was due to a glanders-like organism.

In 1920, he succeeded FRASER as Director of the Institute of Medical Research, Kuala Lumpur.

In 1926, STANTON was selected by the Secretary of State for the Colonies to be the first holder of the newly-created post of Chief Medical Adviser in the Colonial Office, and he continued to hold this post up till the last. His outstanding services in this capacity were recognised by his being made a *C.M.G* in 1929, and promoted to a *K.C.M.G.* in 1934. Mr. ORMSBY GORE had already at the end of last summer invited him to continue to hold his appointment in the Colonial Office for a further period of 3 years from April 1st, 1938, and this invitation had been accepted. After returning to London, he continued to take a great interest in the welfare of the Society, of which he had been Local Secretary while in Malaya. and was instrumental in obtaining large contributions towards the Manson House Fund.

It is not too much to say that STANTON was beloved by everyone with whom he came into contact. No one who met him could fail to be impressed by the combination which he presented of great professional eminence, an equitable character, keen sense of humour and warm kindliness. He never thought evil of anyone, and yet was a good judge of the character of others. In discharging his official and other duties, he was the most conscientious of men, and it may be said that the responsibility of his position caused him far greater anxieties than any but his most intimate associates realised.

The members of the Society have lost a great friend, whom it will be hard to replace.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOL. XXXI. No. 6. APRIL, 1938.

Proceedings of an **Ordinary Meeting** of the Society, held at Manson House, 26, Portland Place, London, W.1, at 8.15 p.m., on Thursday, 17th February, 1938.

Lt.-Col. S. P. JAMES, *C.M.G.*, M.D., F.R.S., I.M.S. (ret.), *President*, in the Chair.

PAPERS.

(1) CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE By H. M. HANSCHELL

(2) CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE IN AFRICAN NATIVES - - - By C. C. CHESTERMAN

(3) CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE—EXPERIMENTAL INVESTIGATION - - By G. M. FINDLAY

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE.

BY

H. M. HANSCHELL, *D.S.C.*, M.R.C.S., D.T.M. & H.,

Hon. Medical Superintendent, Royal Albert Dock Hospital;
Hon. Associated Physician, Hospital for Tropical Diseases, Gordon Street,
(Seamen's Hospital Society, London).

Mr. President, Ladies and Gentlemen, I must first express to you my lively sense of the honour done me by inviting me this evening to open a discussion on climatic bubo. I shall try to confine myself to those lesions which I have dealt with. They are inguinal lesions, and the less serious and the less than half the disease, which when afflicting the woman's vagina and vulva, and the rectum in either sex, is indeed a serious one.

Since 1919, at the Seamen's Hospital, Royal Albert Dock, I have diagnosed 130 cases of climatic bubo in the male, among them representatives of every colour and almost every race. These 130 cases have been studied against controls of 17,900 male cases of lesions of the genital-inguinal area (including gonorrhoea), all also under my care at the same hospital, and among them also representatives of almost all races. Among these 17,900 cases have been many of chancroid, chancre, balanitis, venereal warts, herpes preputialis, penile and scrotal scabies, pediculosis, accompanied by an inguinal adenitis ranging in degree from bubo and abscess to mere enlarged, tender glands—whose infections were acquired in England and for the most part in the dockland areas; yet among these so far no case of climatic bubo has been discovered.

Every case I have seen has been in the male, and with infection acquired in the tropics or sub-tropics. The disease is therefore for me strictly a venereal disease of the tropics. With the clinical experience thus outlined, I may assert that indigenous infection here, must be very rare; for in an area constantly exposed to its importation, no case has come to light in 19 years of watching for it.

The disease now excites among us a widespread interest—because Dr. G. MARSHALL FINDLAY in one of his brilliant researches, conveyed the infection, with production of inguinal bubo, to the guineapig, and showed that the causative

agent was a filter-passer virus, and because of Dr. STANNUS's scholarly researches, which have served so well not clinical alone but psychological medicine too ; for I suspect that after he had exposed it, urbi et orbi, in his famous monograph, now a classic, to be no less than "A Sixth Venereal Disease," then not a few respectable physicians, formerly coldly inattentive, could find in an active interest in its lesions a laudable outlet for all sorts of repressions.

Climatic bubo, Sir, is unique in being a respectable venereal disease. Select nursing homes take it in with murmurs of sympathy, and to the almost sanctuary wards of eminent physicians it is welcomed gladly. Unlike its vulgar congeners, the clap and the pox, the vernacular has no name for it, and so anyone may talk about it and anyone may have it. Nevertheless, it is still a disease of minor importance to the public health in this country.

I trust, Sir, I may be allowed to be a little reminiscent. My interest in lesions of the groin was formed before ever I began as medical student. In *Tristram Shandy* I had followed eagerly that affair between Captain Tobias Shandy—" My Uncle Toby "—and the enchanting Widow Wadman. Uncle Toby, you will remember, Sir, had served with gallantry abroad and there acquired a wound in the groin—and he was extremely bashful about it, and the widow, unknown to him, had thoughts of marrying him, and was extremely inquisitive about it. In those pages I learned what later experience confirmed, that the idea of a lesion in the groin may carry an emotional content worthy even the notice of literature.

My good fortune in my teachers held on. Early in 1910, Dr. CARMICHAEL LOW demonstrated to me in the Albert Dock Hospital a climatic bubo, with no penile lesion, from the China coast in a white ship's stoker. Dr. LOW suggested then that a chronic case ending in fibrosis of all the femoral and pubo-inguinal glands would cause elephantiasis of the leg, and perhaps this might account for some cases of tropical elephantiasis where no filariasis could be postulated.

Dr. LOW said it was acquired in copula. His theory-shattering regard for facts and his diamond common-sense would not permit the history of recent coitus to rank as cogent evidence—" No, no," he said, " they all do it." But he had made a complete clinical examination of a patient in the tropics, detected the papulo-herpetiform primary lesion on under surface of prepuce, noted its rapid disappearance and watched the rise of the inguinal adenitis. It was a venereal disease of the tropics conveniently cloaked as climatic bubo.

Now, Sir, from the simple stoker to the proud pro-consul. Such an one was also a patient in the Albert Dock Hospital in 1910. He accounted for the old scars in his groin by stating that when a young man he had served on the Frontier in India and there acquired climatic bubo. His present condition required that surgeons consult over him. One of them, Mr. President, was a distinguished member of your distinguished Service. After leaving the bedside the surgeons talked, as surgeons do. I kept respectfully in the background, but naturally within earshot. One surgeon was puzzled about this climatic bubo,

and the surgeon from India told him it was a common affection of the European in India, so much so, he said, that a lady out there had asked him how it was that so many officers had been wounded in the groin.

In my 130 cases, the primary lesion was present in only four. It appears 3 to 5 days after coitus and is painless. It is a woeful fact that events beneath a prepuce may go for long, or for ever, unnoticed—but when patients have noticed it they described the primary lesion as a pimple or a sore-blister. It usually disappears in at most 8 days—so I judge from histories. The four I have seen were exceptional in that they were still present 14 to 21 days after first appearance. The swelling in the groin is noticed by the patient 7 to 21 days after the infecting coitus. As in chancroid, so in climatic bubo, the male must be an inefficient transmitter of the infection. In climatic bubo there is rapid healing of the primary lesion, and with increasing painful inguinal adenitis increasing disinclination for sexual intercourse. And probably no transmission of virus in the seminal fluid. If all this be true then we have an explanation of the escape of the London dockland areas from infection ; but it carries the corollary that the primary lesion in the woman's vagina must persist for very much longer than it does on the man's penis. For the disease to take root in this country we must import infected women, or more of them.

Of my 130 cases only two have been in circumcised men. A significant experience against the background of the many circumcised men attending my V.D. Clinic at the same hospital. In the prepuce-bearing man the surface of glans and under-surface of prepuce is delicate, softened by the moisture constantly present, and easily penetrated. The preputial sac is a warm, moist, chamber holding up the epithelial debris we call smegma—a good pabulum, in an ideal incubator, for the cultivation of streptococcus, as can easily be shown—and, I am sure, for the virus of climatic bubo too.

The circumcised have many advantages, among them a lessened liability to climatic bubo, and no drawbacks. As to acquired immunity—I have not yet seen a case with the infection acquired for the second time.

The virus of climatic bubo has been reported as infecting a finger—with consequent adenitis of epitrochlear and axillary glands—as infecting tongue and lip with submental adenitis. That this should occur is to be expected.

Lacking animal inoculation tests—then the diagnosis of climatic bubo rests on clinical grounds.

There might be some difficulty in differentiation between an early syphilitic chancre and the papulo-herpetiform lesion of climatic bubo, but the microscope will reveal spirochaetes in the chancre—or watching the case will settle the diagnosis. The chancroid ulcer is characteristic. When we come to the buboes —there are difficulties in individual cases. Comparison of numbers of chancroid buboes and climatic buboes shows that in climatic bubo, peri-adenitis is a more marked feature, there is less pain, fever more common, less suppuration, which tends to be at several points and is not frank and in one area as in chancroid, the

buboes usually far more chronic, they do not so often break down with fistula formation as in chancroid buboes. A healed primary lesion of climatic bubo leaves no trace. A healed syphilitic chancre little if any trace. A healed chancroid ulcer, a sharp precipitous edge scar. In syphilis the adenitis is small in size and there is no peri-adenitis—except in the rare case where the primary chancre is also septic. Even if the climatic bubo case give positive Wassermann and Kahn blood tests—anti-syphilis treatment has no effect on the bubo.

Lastly, there is the intradermal test introduced by FREI. I am here very much indebted to Dr. MARSHALL FINDLAY for his carrying out of animal inoculations for me, and to Mr. A. H. WALTERS who, at the Albert Dock Hospital, has prepared the antigens and has kept a daily, often hourly, critical watch and record of their effect when injected intradermally. It is, in fact, due to his initiative and persuasion that we started observations on the Frei skin test. A true positive, we claim, should produce besides the marked erythema a nodule in the skin—FISCHER'S " Knotchen"—which should persist for at least 2 days.

Three batches of antigen were made according to the methods described by FREI and HOFFMANN, and, following them, used by HELLERSTRÖM. Each batch was prepared from material obtained from a separate patient with a climatic bubo : but no animal control inoculation for presence of active virus. Two of these batches failed consistently. One batch gave fairly good results for about 6 months and then failed consistently. These three batches were tried against another, a pooled antigen. I excised affected inguinal glands from selected cases. The excised gland was bisected at once : one half was preserved by Mr. WALTERS, the other half submitted to Dr. FINDLAY for animal inoculation. By his finding active virus, we were able to select three batches of half-glands from three separate cases as known to be infected with the virus—and from these three batches Mr. WALTERS prepared a pooled antigen. We have found that such a pooled antigen gives true positives 3 years after preparation—and that such a pooled antigen will give positive Frei tests in 95 per cent. of cases of climatic bubo. Here again a positive in our reckoning means besides a well-marked erythema persistence of the nodules.

The control intradermal test has been carried out always with Dmelco's chancroid vaccine. This pooled antigen has been tested for control—on thirty-one cases of sero-positive syphilis with palpable, and in some of them visible, inguinal adenitis : twenty-eight gave completely negative results, three gave rather well-marked erythema but no nodules. As against this, six cases diagnosed as climatic bubo with persistent Wassermann and Kahn reactions all gave positive results. Then five cases of microscopically demonstrated gonococcal urethritis accompanying climatic bubo all gave a positive result. In a series of forty gonorrhoea cases in the male none gave a nodule : only three gave a more or less well-marked erythema alone. Twelve consecutive cases of chancroid cum bubo gave negative results. None gave even marked erythema. Of four

cases of venereal warts, unaccompanied by gonorrhoea, two gave erythema, rather well marked, and no nodules.

My material has not allowed of observation as to how early after infection a positive skin test may be obtained—it may be got, however, 21 days after exposure to infection, and may persist for months. In one of my cases we found it still present 12 months after discharge from hospital—that is, 16 months after exposure to infection. In three of my cases of climatic bubo, using the pooled antigen, a negative result, that is an erythema only, was obtained 21 to 30 days after exposure to infection—but later, 42 to 51 days after infection, the same antigen on these same cases gave a positive result, that is erythema and nodules. In some cases of climatic bubo the pooled antigen has given repeatedly negative results. With this experience I must submit that no verdict should be given on result of the skin test alone, and the test should be repeated. Obviously antigens from different hands and different patients must vary widely as to potency and as to persistence of potency, and there is no agreement yet as to what constitutes a true positive, a negative, and a doubtful result. Standardisation is needed, and probably this can be attempted only by work in the laboratory and by animal inoculation. I submit it may be urgent—for a spate of cases, in perhaps great clinical variety, " proved " by " positive " skin tests may soon descend on us. And, what is the greater pity, though the lesser irk, true cases may be rejected on account of " negatives " from faulty antigens.

Treatment of Climatic Bubo.

In many cases there is rapid subsidence of the buboes after a few days' rest in bed with no other treatment. If there be fluctuation pus should be aspirated and puncture sealed, with all aseptic care. The bubo should not be incised. Intravenous injections of T.A.B. vaccine hasten resolution of the adenitis. In some 85 per cent. of cases no other treatment has been given, and after 4 to 6 weeks the patients have been discharged. But there remain the 15 per cent. in whom operation is advisable. It is true that in time, in all but a very few, complete recovery will take place—but this may mean many months. So that as a working rule, if after a fortnight there is no sign of quick, progressive, recovery—excision of the affected glands should be considered.

Operation often discloses that apparently but slightly enlarged glands contain many very small foci of pus—that the glands may have a small central core of lymphoid tissue, sometimes showing points of pus, surrounded by a dense fibrous wall. These glands could never recover. The operation is not one for the rash or the fumbling. The glands are adherent often to the spermatic cord, the femoral vein, the external iliac vein.

There is no satisfactory evidence that any drug has any effect on the disease —though to-day if one should find or suspect an added infection of streptococcus the indication for sulphanilamide or sulphonamide compound would be obvious.



FIG. 1.—Papulo-herpetiform primary lesion appeared 5 days after infecting coitus in South America ; still present on 26th day but had disappeared by 30th day without any treatment. No *Spirochaeta pallida* detected.

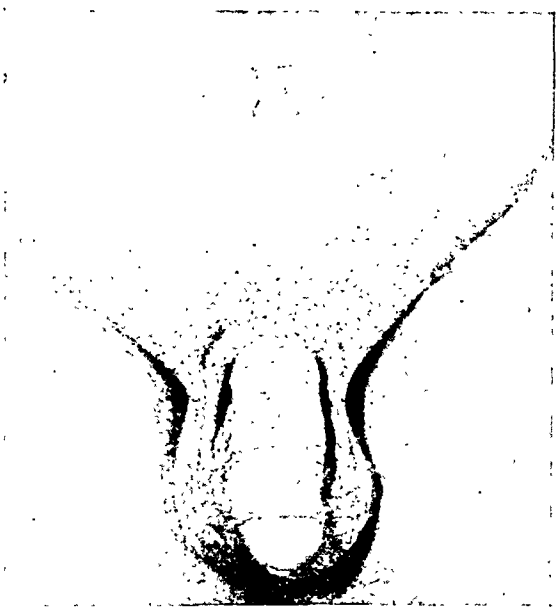


FIG. 2.—Twenty-six days after infecting coitus in South America. Frei test positive. Wassermann and Kahn tests negative.

CASE 120.—CLIMATIC BUBO.

Sulphanilamide (Bayer-Prontosil), in my hands, has so far always procured rapid healing of chancroid ulcers and buboes, quite apart from added streptococcus infection. These compounds are bound to be tried on climatic bubo.

I now report briefly a case recently occurring in my private practice. An Englishman, married, 38 years old, consulted me for a supposed syphilitic rash on the body, and a discharge from the rectum accompanied by burning and itching. The rash was pityriasis rosea. No lesions on penis or genital area and no adenitis. Blood Wassermann and Kahn tests, repeated once, negative. The anal margins were moist with a thin, sticky, faintly yellowish discharge. Proctoscopy revealed just above the line of the anal papillae a number of pearly-looking bodies, like sago grains on a red base, situated on the lowest centimetre of the columns of Morgagni. They reminded me of the sago body urethritis sometimes disclosed by urethroscopy of the male urethra. The patient gave a very strongly positive Frei test with the pooled antigen already described. The test was repeated after 3 weeks and was again positive.

I was now able to pursue tactfully but persistently further enquiries. I elicited that he had, about 6 weeks before seeing me, engaged in sodomy with a young European in Egypt. My patient had been both active and passive agent. A few days later he had gone to see the younger partner again, and found that he had been put to bed in a clinic, for, it was stated by the servant, a swelling in the groin, the result of a strain from riding a new pony. About a fortnight later still my patient resumed marital intercourse per vaginam et per rectum.

I was able to take samples of blood and also to carry out the Frei test with the same antigen on his wife, but did not ask for a physical examination. Her Wassermann and Kahn tests were negative, the Frei test was also negative, and she denied noticing anything wrong with vagina or rectum. Digital examination per rectum of the husband did not reveal any thickening of the rectal wall, nor could any peri-rectal tumour be felt. No gonococci, no spirochaetes, no ova, protozoa or dysentery bacilli detected on examination of the rectal discharge.

After 5 weeks' use of rectal suppositories containing a bismuth paste nearly all lesions had disappeared. I have not seen him again. Probably this is an early case of direct infection of the rectal mucosa with the virus of climatic bubo.

I must register my agreement with Dr. STANNUS in objecting to the nomenclature. Climatic bubo is far too narrow and carries with it a false implication : for the bubo is but the less serious part of the disease, which is not climatic but world-wide. And lympho-granuloma inguinale is confusing—with other different and distinct lesions. I support him in his advocacy of the term—Poradenitis.

In conclusion, I must express my deep indebtedness and my gratitude to Dr. G. MARSHALL FINDLAY for his direct and valuable help, and for his researches into the aetiology of this infection—and in equal degree to Dr. STANNUS, who opened for me a clearer and wider window on this disease. Dr. STANNUS in person has indeed shown us that unrelenting research in the library by one long

trained in clinical medicine, whose distinction in that field is unquestionable, may render to Medicine services no less signal than those given by research at the bedside, by necropsy, or at the laboratory bench. Lastly, I record my thanks to Mr. A. H. WALTERS for his assistance with my cases. His skill in making the antigens, his accuracy in watching and recording, and his demonstrating of the skin reactions to me—for long very sceptical—have convinced me of their value.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE IN AFRICAN NATIVES.

BY
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All I can hope to add to this discussion is some information as to the incidence and symptomatology of this condition in the Belgian Congo, where in most urban centres and in some rural areas foci of the disease exist.

My interest in the syndrome was first aroused by listening to the paper read by Dr. H. S. STANNUS (1932)* to the Royal Society of Medicine, in which he stated his conviction that cases would be found if looked for among native women in Central Africa.

I realise that it is a very dangerous thing to look for anything because one is sure to find it, but a subsequent search by myself and my colleague, Dr. R. E. HOLMES, at the hospital of the Baptist Missionary Society, Yakusu, near Stanleyville, revealed a widespread incidence among women patients of the prostitute class.

After one of the first cases of rectal stricture had benefited by dilatation, a considerable number presented themselves. The extremely unpleasant symptoms resulting from the chronic progressive ulceration and fibrosis, drive sufferers to avail themselves of any prospect of relief, which, unfortunately however, they generally fail to obtain.

Cases of climatic bubo, in white and black males, had frequently been recognised at the hospital where soft sore, syphilis, septic and filarial inguinal adenitis are commonly seen.

Frei's skin test has proved itself as reliable as it is useful.

In the absence of facilities for Wassermann or Kahn tests for syphilis, strong confirmatory evidence of that disease was afforded by a negative Dmelcos and Frei test in any case of bubo with a history of genital sore. Two cases in males illustrate the commonly observed aetiology and symptomatology among blacks. It should be noted that practically all males are circumcized in this area.

CASES IN NATIVE MALES.

Case 1.—*Sailor, aged* 40. Eight days after coitus with a prostitute, noticed a pimple on coronal sulcus, 15 days later left inguinal bubo burst. He was seen 8 weeks after infection with a fistula in left groin and a softened bubo in right groin. He was also suffering from trypanosomiasis but after sterilising treatment with Bayer 205 and tryparsamide the pus from the right bubo was aspirated and used for the making of Frei's antigen. The aspirated bubo healed but the other remained fistulous.

* STANNUS, H. S. (1932). *Proc. R. Soc. Med.*, 26, 7-16.

Case 2.—*Male, aged* 25 (see Plate). Seen 1 year after attack of inguinal adenitis. Had not noticed primary sore. Typical puckered scar in groin with three fistulous openings. Fibrosis and deformity at root of scrotum with three fistulae. Treatment ineffective.

The Syndrome in Native Women.

I have notes of the ten cases diagnosed in 1933. The following is a résumé of the salient features.

(1) *Rarity of inguinal adenopathy:* one case in ten where the primary sore was on the fourchette. Healing occurred without fistulation.

Another case with the primary lesion observed anteriorly escaped without the slightest inguinal adenitis, although her husband (Case 1 above), by whom she had been infected, had typical climatic bubo.

(2) Frequency of association of rectal stricture with vulval lesions, i.e., in five cases out of the six in this series whose infection had lasted more than 2 years. The stricture is formed between 4 and 7 cm. from the anus, and was generally accompanied by ulceration and a purulent discharge with ribbon-shaped faeces. Multiple fistulae often develop in the perinaeum and buttocks.

(3) Three showed ano-vaginal fistulae, of $\frac{1}{2}$ to $1\frac{1}{2}$ cm. diameter. An attempt made to close one of these surgically proved unsuccessful, the sutures breaking down a week later.

(4) The primary lesion is generally an ulcer on the labia or vestibule. The subsequent evolution of the local lesion lends itself by its variety to flights of descriptive eloquence and symbolic imagery. The essential factor is chronic ulceration with little epithelialization, together with surrounding induration. Thus *mucous surfaces* come to resemble turgid cockscombs with serrated edges, perforations or adhesive bands. I have seen the latter at the urethral orifice causing stricture.

Skin covered parts become elephantoid with verrucose and fissured surfaces resembling relief maps.

Reports from other Mission hospitals in the Congo confirm our experience of the frequent occurrence of this disease. In some it appears to be as frequent as gonorrhoea.

Clinicians called upon to deal with such cases would welcome :—

(1) A reliable supply of Frei's antigen which could be kept in stock. Suitable cases of climatic bubo are rare, and do not as a rule furnish sufficient antigen for more than a few tests.

(2) A specific remedy for this virus disease.

Amputation of elephantoid excresences or dilatation of rectal stricture is the utmost one can do to relieve, and recurrences frequently follow.

The syndrome causes a great deal of misery and disability in native women, but represents only a part of the major problem of venereal disease among primitive negro races.



FIG. 1.—CLIMATIC BUBO. One year after infection

Note—(1) Depigmented spot on coronary sulcus, probable site of primary lesion.
(2) Typical puckered scar in groin with three fistulae.
(3) Distortion and induration of scrotum with three fistulous openings at root.

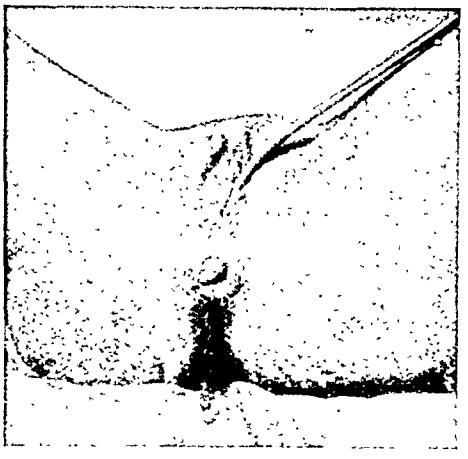


FIG. 2.—ANO-GENITO-RECTAL SYNDROME.

Note—(1) Catheter traversing ano-vaginal fistula.
(2) Indurated peri-anal mucosa (not haemorrhoids).
Rectal stricture was present.



FIG. 3.—ESTHIOMENE WITH ELEPHANTIASIS VULVAE.

Note—(1) Verrucose surface of elephantoid tissue. Swab passed through perforation in left *labium minus*.
(2) Elephantiasis of clitoris.
(3) Partial atresia of vaginal orifice.

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TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

CLIMATIC BUBO OR LYMPHOGRANULOMA INGUINALE—EXPERIMENTAL INVESTIGATION.

BY

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The disease, varyingly referred to as climatic bubo, lymphogranuloma inguinale, lymphogranuloma venereum, lymphopathia venereum or poradenitis has in the past few years received very intensive study. As a result, it is now realized that in man lymphogranuloma inguinale, to use the name most generally employed, is not restricted merely to a primary lesion in the genital region and a suppurating bubo involving the inguinal lymph nodes, nor even to such changes as stricture of the rectum, stricture of the vagina and elephantiasis of the vulva, penis and scrotum. Lymphogranuloma inguinale, in fact, is now recognized to be a generalized disease with such protean symptoms that in its early stages it may even suggest typhoid fever. Arthritis and skin rashes are not uncommon, while conjunctivitis and meningitis may occur. In addition characteristic biochemical changes have been recorded in the blood, such as a decrease in the lipin content, an increase in the percentage of free cholesterol (ROSEN, ROSENFELD and KRASNOW, 1937) and a hyperproteinaemia with increase in the globulin and decrease in the albumin content of the serum (WILLIAMS and GUTMAN, 1936).

Since this somewhat changed conception of the nature of lymphogranuloma inguinale has occurred as a result of the successful transmission of the disease to laboratory animals and the demonstration that the causal agent is a filterable virus, it may not be without interest to review very briefly what is now known of the experimental disease in animals and of the nature of the virus.

The Transmission of the Disease to Animals.

Although from time to time claims had been made that lymphogranuloma inguinale could be transmitted to guineapigs by injection into the groin of pus from human inguinal buboes (*cf.* Gamna, 1923) the first definite evidence that the aetiological agent is a filterable virus was obtained by Hellerström and Wassén (1930) who showed that the intracerebral injection of certain species of monkey with material obtained from human lymph nodes infected with lymphogranuloma inguinale gives rise to a meningo-encephalitis which can be transmitted in series, after filtration through Berkefeld V or Chamberland L_3 candles. Although the rhesus monkey, *Macaca mulutta* is relatively insusceptible, the infection is readily transmissible to *M. inuus*, *M. cynomologus*, *Cercopithecus æthiops*, *Cercocebus fuliginosus*, *Cynocephalus babuin*, *Cebus fatuellus*, *Callithrix jacchus* and *C. penicillata*. In addition to intracerebral inoculation, monkeys can also be infected by injection into the prepuce, when the inguinal lymph nodes become enlarged, into the peritoneal cavity, perirectal tissues (*cf.* Findlay, 1936), and by placing infected material on the scarified skin. Inoculation of infected material on to the scarified cornea does not set up a keratitis but injected into the anterior chamber of the eye there occurs partial corneal opacity and iridocyclitis followed by atrophy of the eyeball.

After intracerebral injection in monkeys the virus may find its way into the blood stream and reach the liver, kidney, spleen and inguinal lymph nodes. There is no evidence that the infection travels either centrifugally or centripetally by nerves.

The discovery by Levaditi and his collaborators (1932), confirmed by Findlay (1932), that the virus of lymphogranuloma inguinale can be transmitted to mice by intracerebral inoculation has been followed by an intensive study of the distribution of the virus of lymphogranuloma inguinale in various human lesions. In this country twenty-six strains of the virus have been successfully transmitted to mice by intracerebral injection, the amount injected being 0·03 c.c. of a 20 per cent. suspension of material. The pathogenicity of different strains, as previously pointed out (Findlay, 1933), shows considerable variation. Two of the above twenty-six strains have proved highly virulent since they give rise to cerebral symptoms in mice 2 to 4 days after injection and show no tendency to decrease in virulence. Other strains, after a variable number of intracerebral passages, have lost their pathogenicity for no apparent

reason. It is sometimes possible to enhance the virulence of a strain by using for inoculation suspensions relatively rich in elementary bodies, such suspensions being prepared by differential centrifugation, as has been done in the case of vaccinia elementary bodies.

The symptoms seen in mice consist of roughening of the coat, hunching of the back and, in some animals, a purulent conjunctivitis, followed in a few days by death.

After intraperitoneal inoculation, associated with injection of starch into the brain, the mice may die with meningitic symptoms (FINDLAY, 1933; WASSÉN, 1935): if the brain is not traumatized cerebral localization does not occur.

Apart from monkeys and mice, meningitis has been produced in the cat (LEVADITI, *et al.*, 1932), the dog (FINDLAY, 1933) and in rodents such as the field vole *Microtus agrestis*, the spermophile *Citillus citillus* and the striped ground squirrel *Eutamias orientalis*. Intracerebral inoculation of guineapigs results in meningitic lesions in only a small percentage of animals, while intracerebral injection of rabbits and rats is even less successful. Claims to have produced meningo-encephalitis in rabbits have largely failed to take into account the frequency of spontaneous rabbit encephalitis. The virus can, however, survive for 10 or more days in the brains of rabbits but there is no evidence that the virus increases during this period.

The production of buboes in the groins of guineapigs by subcutaneous injection of lymphogranuloma inguinale material has already been mentioned. Although such buboes can be produced with many strains of the virus it is not easy to transmit the disease in guineapigs' groins for more than a few passages and in any case the buboes involving the lymph nodes, which appear about 2 days after inoculation, only remain palpable for from 10 to 14 days after injection. The disease thus tends to be a self-limiting infection. Occasionally, however, there may be enlargement of the lymph nodes in the pelvis and mesentery. NICOLAU (1932) has found that buboes may also be caused in dogs by injection of infected material into the groin. VON HAAM and HARTWELL (1937) have given rise to a virus pneumonia in monkeys and mice by intranasal instillation of infected tissues.

In addition to transmission of the infection by means of pus or excised tissues from the inguinal lymph nodes, RAVAUT, LEVADITI, LAMBLING and CACHERA (1932) and LAEDERICH, LEVADITI, MAMOU and BEAUCHESNE (1932), by employing an ingenious technique, isolated from inflamed rectal tissue a virus which agreed in every way with the virus isolated from inguinal buboes. The method employed by these observers consisted in implanting a portion of inflamed rectal tissue into the groin of a guineapig and then after a few days removing the corresponding inguinal lymph nodes which, though bacteriologically sterile, contained the virus. The strain of virus isolated by MOCQUOT, LEVADITI and REINIÉ (1935) from an inflammatory condition of the colon is of interest, as with it LEVADITI, MOLLARET and REINIÉ (1935), produced the histological changes

characteristic of lymphogranuloma inguinale in the rectum of chimpanzees by direct inoculation into the mucosa, while in human volunteers they reproduced the typical primary penile sore followed by involvement of the inguinal lymph nodes. The virus has also been transmitted to animals by material from a case of conjunctivitis (LEVADITI, BOLLACK, BASCH and DESVIGNES, 1936), while VON HAAM and D'AUNOY (1935) have obtained it in two cases from the cerebrospinal fluid.

PATHOLOGICAL CHANGES.

The primary skin lesion shows an infiltration with mononuclear leococytes, plasma cells, plasmacytoid cells and a few polymorphonuclear leucocytes. The infiltration is most marked in the dermis and seems to begin in the perivascular lymph spaces. The endothelial cells of the blood vessels swell up and eventually the lumen of the vessels becomes obstructed, leading to a local necrosis of the epidermis.

The histological changes in the infected lymph nodes have been frequently described. In the earliest stage there is congestion of the vessels and accumulations of lymphocytes and mononuclear cells, derived from the germinal follicles which are in a state of active mitosis and from the reticulo-endothelial cells of the node, together with occasional giant cells and polymorphonuclear leucocytes. At the same time the fibrous stroma begins to proliferate. In a very short time the smaller trabeculae break down and small co-called stellate abscesses are dotted through the gland substance. If the nodes are near the surface the whole mass may then break down but if, as WALTERS (1935) points out, the nodes are situated deeply the proliferated connective tissue may be sufficiently developed to enclose the necrotic area which is eventually replaced by fibrous tissue. In monkeys and guineapigs the earlier stages of bubo formation are well seen with the formation of stellate abscesses, but actual breaking down of the gland substance does not occur. In monkeys, giant cells are not found although in guineapigs, cells containing three or four nuclei are occasionally present among the inflammatory cells.

In the brains of animals injected intracerebrally with material containing the virus of lymphogranuloma inguinale the lesions are largely confined to the meninges, more especially the pia mater and subarachnoid spaces. The virus of lymphogranuloma inguinale does not in fact produce a true encephalitis since neither the nerve cells themselves nor the neuroglia show any direct reaction. The most characteristic reaction in the central nervous system is the presence of masses of cells in the Virchow-Robin space round the blood vessels and in the meninges; these cells are collected into definite nodules, closely resembling the areas found in lymph nodes. In the adrenals of monkeys, collections of lymphocytes, plasmacytoid cells and occasionally polymorphonuclear leucocytes may be seen. These observations are of interest in view of the claims that have

been made that in man lymphogranuloma inguinale may on occasions involve the adrenals and kidneys (*cf.* REICHLE and CONNOR, 1935, and DORÉ, BREUIL and LAFFERRE, 1936).

In connection with the experimental production of a fatal meningitis in animals it may be of interest to record the fact that a number of human cases have been reported with symptoms of meningitic involvement. CHEVALIER and BARNARD (1932), for instance, described chronic meningismus in a woman with lymphogranuloma inguinale : the cerebrospinal fluid, which showed the presence of an excessive number of cells and albumin, gave a positive Frei reaction when injected intradermally. MIDANA and VERCELLINO (1934) reported two similar cases. DAVID and LORING (1935) described epileptiform convulsions in a patient in whom the ano-rectal lesions had extended to the colon, while RAJAM (1936) recorded a fatal case of meningitis in a patient with lymphogranuloma inguinale. Here again the cerebrospinal fluid gave a positive Frei test on intradermal injection. There is thus little doubt that as is the case in experimental animals lymphogranuloma inguinale may involve the human central nervous system. Similar nodules to those found in the central nervous system are also produced in the chorio-allantoic membrane of the developing chick embryo. Fuller accounts of the pathological changes produced experimentally by lymphogranuloma inguinale are given by FINDLAY (1933), WASSÉN (1935) and LEVADITI (1936).

It will thus be seen that the essential lesion produced by the virus of lymphogranuloma inguinale is an involvement of lymph spaces and lymph channels. The particular lymph nodes and lymph channels involved depend on the site of inoculation and, since the virus is transmitted almost entirely by the lymphatics, on the distribution of the lymphatics leading from the site of inoculation. The extensive reaction associated with blocking of the lymphatics probably causes a secondary reaction from lymph stasis and at the same time explains how it is that infected tissues may become shut off from the immune bodies present in the blood serum. Virus may thus remain active for long periods, despite the presence of virucidal immune bodies in the serum. CAMINOPETROS (1935), for instance, has found virus present in the vagina at least 18 months after infection.

THE VIRUS.

Bodies which, it was suggested, might be of aetiological significance were first described by GAMNA (1923) and FAVRE (1924) in the cytoplasm of certain of the cells from inguinale buboes. These bodies are from 1 to 4μ in size and in many cases stain positively by the Feulgen technique. FINDLAY (1933) pointed out their non-specific character since similar bodies were described by FLEMMING (1885) in cells from normal lymph nodes.

The credit for first describing what is almost certainly the causative agent goes to GAY-PRIETO (1927), who mentioned the presence of small cytoplasmic granules in the cells from inguinal buboes 1μ or less in diameter and often occurring in small clumps. These granules were also described and figured by FINDLAY (1933, Plate IV, Figs. 7 and 8), who suggested the possibility that they represented the virus. This possibility has now been amplified, more especially by the work of MIYAGAWA and his colleagues (1935 and 1936). The granules in stained preparations appear either singly, in pairs or in short chains, sometimes arranged to form a circle. Certain of the diplococcal forms suggest division. Less commonly the granules appear as dense clumps, comprising some hundreds of minute bodies. Studies are now in progress in collaboration with my colleagues, Dr. R. D. MACKENZIE and Dr. F. O. MACCALLUM, to determine whether these particles pass through a cycle in any way comparable to that of the psittacosis virus.

The evidence that these granules actually represent the causal agent of lymphogranuloma inguinale may be summarized as follows :—

(1) The presence of the granules in the lesions of lymphogranuloma inguinale in man, in the brains of infected mice and monkeys and in experimental buboes in guineapigs.

(2) The presence of similar granules in the chorio-allantoic membrane of the developing chick embryo infected with lymphogranuloma inguinale and in the cells of the rabbit's cornea grown in tissue culture (NAUCK, 1937).

(3) The presence of granules in Tyrode-tissue cultures of the virus of lymphogranuloma inguinale (*cf.* TAMURA, 1934 and 1937).

(4) The dimensions of the virus as shown by filtration through graded collodion membranes are similar to those of vaccinia virus, namely 0·125μ to 0·175μ (MIYAGAWA, *et al.*, 1935[2]; and BROOM and FINDLAY, 1936).

(5) An anti-serum prepared in rabbits from which the elementary corpuscles have been removed by differential centrifugation contains no virucidal immune bodies: on the other hand an anti-serum prepared by the infection of relatively concentrated suspensions of elementary bodies, relatively free from tissue elements, contains virucidal immune bodies. It thus appears that the presence of elementary bodies is essential for the production of virucidal immune bodies.

Experiments are at present in progress to determine the relationship between the elementary bodies and the production of agglutinins and complement fixing immune bodies.

DIAGNOSIS.

Various methods have been devised for the diagnosis of infections due to the virus of lymphogranuloma inguinale. The most important is of course the isolation of the virus by intracerebral injection either of monkeys or mice.

In addition, however, a number of diagnostic tests have been based on the immunological changes characteristic of the disease.

(1) *The allergic test*, originally described by FREI (1925) is now well known. The antigen may be prepared either from the pus or the nodes of an inguinal adentitis or from the brains of infected animals (monkeys or mice). The chorio-allantoic membrane of the developing chick embryo may also be employed and has the advantage of eliminating extraneous viruses which may be contained in mouse or monkey brains. PAULSON (1937 and 1938) has recently obtained positive Frei tests with material obtained by aspiration from the inflamed mucosa in cases of ulcerative colitis of undetermined aetiology, the material before heating being diluted 1 in 10 with azochloramide, thus confirming and extending the observation of NICOLAS, FAVRE, LEBEUF and CHARPY (1932), who obtained positive skin tests with an antigen prepared from pus removed from a fistula associated with rectal stricture. The test is now generally regarded as highly specific though it has been suggested that the brains of apparently normal mice may produce a false positive when injected intra-dermally. The number of persons who are sensitive to mouse brain proteins is, however, exceedingly small. Among 2,000 persons injected for other purposes with mouse brain tissues only two showed any reaction at the site of inoculation. The chief difficulty in regard to the use of the Frei antigen lies in the fact that the activity of the extract cannot be easily standardized and that the activity decreases with keeping even in the ice chest. Some samples, however, show little decrease in activity even after 18 months, while other antigens become inactive after only 2 or 3 months. Therefore, unless the material to be injected can be regularly tested on the skin of a person known to react positively, a negative reaction may be due not to absence of infection in the individual but to failure of antigenic potency in the material injected.

Occasionally when the intradermal injection of 0·1 c.c. of the antigen is negative the injection of 0·3 c.c. may produce a positive result.

Very little work has been carried out on the factors underlying the Frei test. If the antigen is filtered through a Berkefeld V filter the filtrate is still capable of producing a positive reaction but passage through a Seitz K filter which removes the greater number of elementary bodies also greatly reduces, or more commonly inhibits, the antigenic capacity of the filtrate.

A similar association between the presence of elementary bodies and activity in the Frei antigen is shown by the following centrifugation experiment. Active Frei antigen was centrifuged at 15,000 r.p.m. for 1 hour; by this means all elementary bodies are thrown down and if the extract had not already been heated infectivity would have been removed from the supernatant. The supernatant fluid was pipetted off, the sediment washed in saline, recentrifuged at 15,000 r.p.m. for 1 hour, and the sediment resuspended in saline and centrifuged for 15 minutes at 3,000 r.p.m. This second supernatant was then pipetted off and the sediment discarded. The first or original supernatant fluid

which failed to show the presence of elementary bodies failed also to give positive intradermal test. The second supernatant showed the presence uniform granules corresponding in size to those of the elementary bodies a in addition gave a positive Frei test.

It is thus possible to obtain a positive Frei test with a killed suspensi of washed lymphogranuloma inguinale elementary bodies just as in the case vaccinia, as shown by CRAIGIE and WISHART (1933), an intraderm allergic test can be obtained with killed suspensions of vaccinia elementa bodies. It, therefore, appears that the Frei test is an allergic reaction d to the presence of lymphogranuloma inguinale elementary bodies in the mater injected. It is of interest to note that occasionally repeated intradermal injectio of Frei antigen may give rise to a hyperallergic condition as in the case of patient described by NICOLAU (1937) where reddish brown papules appear on both arms 2 days after the injection of antigen into one arm. Accordi to SAENZ (1935) exposure to X-rays may also increase the allergic response in t skin. The exact length of time during which the Frei test remains positi in patients who have recovered from lymphogranuloma inguinale infectio has not been accurately determined. In human cases experimentally infecte with lymphogranuloma inguinale, WASSÉN (1935) found that the Frei te becomes positive in from 6 to 13 days after infection and in three persons was still positive 398, 309 and 280 days later; in two others, however, the tes though positive 41 days after infection, was negative 109 and 116 after infectio In cases of involvement of the rectum the Frei test apparently remains positi for some years, a finding which may possibly be correlated with the continue presence of the virus in the granulomatous lesions.

Experiments to determine how far a positive test depends on the continue presence of virus in the tissues in man would be of considerable interest.

Histologically the reaction produced in an infected person by the Fr test reproduces exactly the appearance found in the primary lesion.

In addition to the Frei reaction a number of other tests have been devise for the diagnosis of lymphogranuloma inguinale. REIS (1934) believed tha when the Frei antigen was injected mixed with an equal quantity of the patient' own serum the reaction was intensified, while the intradermal inoculatio of the patient's serum not infrequently gave rise to an allergic reaction simila to that induced by the Frei antigen. Attempts to confirm these observation have failed.

(2) *The guineapig intradermal test.* This was described by WASSÉN (1934 an 1935) and consists in the intradermal injection into the guineapig of a mixture o virus-containing material, such as infected mouse brain and the patient's serum as controls, virus and normal serum and virus and a known immune serum ar similarly injected intradermally. At the end of 48 hours the point of injectio of virus and normal serum is marked by a small red indurated area, the centr of which breaks down to form a tiny ulcer, closely resembling, both macro-

scopically, and microscopically, the primary sore of lymphogranuloma inguinale. The point of inoculation of virus and immune serum shows no reaction. Although this test has the disadvantage that it necessitates the maintenance of a strain of the lymphogranuloma inguinale either in mice or monkeys, its accuracy is probably greater than that of the Frei reaction.

(3) *The intravenous injection of Frei antigen.* This was found by HELLERSTRÖM (1932) to give rise to a febrile reaction in persons suffering from lymphogranuloma inguinale. RAVAUT and his colleagues (1932) have used this reaction in the diagnosis of the disease. Apart from the fact that the absolute specificity of the test is not yet established, the intravenous injection of antigen would seem not to be without possible dangers.

(4) *The intracerebral test in mice.* In this test a mixture of one part of a 10 per cent. suspension of infected mouse brain and two parts of the serum to be tested is inoculated intracerebrally into mice in doses of 0·03 c.c. A control batch of mice is similarly inoculated with a mixture of normal serum and infected mouse brain. With an active virus the mice injected with virus and normal serum should develop sympyoms in from 5 to 10 days.

(5) *Complement fixation.* Efforts have been made by a number of observers to obtain complement fixation using a variety of antigens such as infected human inguinal lymph nodes, infected mouse and monkey tissues. In certain known cases of lymphogranuloma inguinale good complement fixation has been obtained but in others the results have been negative. Owing to these irregularities complex fixation is not a reliable test though it is possible that the infected chorio-allantoic membrane of the developing chick embryo may provide an antigen capable of yielding more consistent results.

(6) *Agglutination.* As the virus particles of lymphogranuloma inguinale are approximately the same size as those of the virus of vaccinia, efforts are now being made to develop a flocculation test on the lines of that used in the diagnosis of variola.

By means of the above tests, as well as by isolation of the virus in experimental animals, it has been possible to demonstrate that in addition to cases imported from abroad autochthonous infections with the virus of lymphogranuloma inguinale undoubtedly occur in this country both in the form of poradenitis (STANNUS and FINDLAY, 1933 and ANWYL-DAVIES, KING and FINDLAY, 1933), or of rectal stricture (FINDLAY, 1936; MANSON-BAHR, 1936; and WHITTAKER, 1937).

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DISCUSSION.

Dr. H. S. Stannus : The three papers to which we have listened this evening have been particularly interesting as the authors have treated the subject from three different points of view. Dr. HANSCHELL has given us his personal store of experience from which one might be inclined to believe that this virus infection produced a disease of trivial and benign character. Speaking rather as the biographer of the subject than as one with much experience of the disease, I think it is perhaps as well to lay emphasis upon the fact that this is not always the case. It seems possible as you have heard from Dr. MARSHALL FINDLAY, that the virus may attack many parts of the body including the meninges, while the general symptomatology may be very varied. It is an infection worth carrying in one's mind when presented with some odd and unusual condition.

With regard to the stricture of the rectum usually described as " inflammatory stricture of the rectum," I feel convinced that all cases are due to this infection. I have been unable to satisfy myself that any single case advanced in the literature as due to syphilis or gonorrhoea was in fact due to either of those diseases. In the female, the infection passes from a primary lesion commonly on the posterior wall of the vagina or fourchette to the pararectal tissues; in the male, the condition is generally associated with a lesion in the anal region due to the practice of sodomy. Apparently rare in this country it is not uncommon on the Continent and in the East.

The swollen perianal tags, which may appear, have before now been mistaken for piles. They are exactly comparable to a similar condition of the vulva, more particularly of the nymphae and are best described as having a cock's comb-like appearance as mentioned by Dr. CHESTERMAN. Genito ano-rectal disease is common among native races and Dr. CHESTERMAN'S experience has been that of many others working among indigenous races. Dr. HANSCHELL'S observations upon the rectal case recently under his care are most instructive. The sago-grain condition of the mucous membrane suggests that described by NAUMAN (1931) about the vulva of a Haitan prostitute. The necessity of a reliable antigen for performing Frei's test must have been realised by many during the last few years, and it is hoped one will be put on the market soon.

The question of treatment still remains a matter of some difficulty; the simple case responds quickly to simple means, but the difficult case may defy all methods. I should be interested to learn whether anyone present has had any experience with sodium salicyate in 6–8 gramme doses per diem by mouth (strict milk diet) combined with anthiomaline (antimonio-thiomalate of lithium) intravenously.

The distribution of the malady is a little difficult to explain. It seems probable that the disease spreads from single foci sometimes as appeared to be the case in Roumania among medical students. I have seen three patients whom I suspect all gained their infection from a woman who parades the Charing Cross Road, in London.

Dr. Manson-Bahr: The speakers have referred to the widespread nature of lymphogranuloma inguinale infections which has become quite an important subject in consulting practice. I have actually seen it, in the form of an acute bubo, being mistaken for plague in England and, of course, when met with for the first time its alarming appearance makes such a mistake possible. There is some reason, too, for believing that in former days in Hongkong lymphogranuloma inguinale was popularly regarded as a mild form of plague and was generally known as *pestis minor*. Of course, this had its social advantages for the sufferer from *pestis minor* was apt to be regarded as a national hero rather than a social pariah.

I have seen cases of lymphogranuloma inguinale with high remittent temperatures which have persisted for several weeks and which have been regarded as typhoid before the buboes in the groin became apparent.

I have also seen cervical adenitis on more than one occasion which has been due to the lymphogranuloma virus and which has given a positive Frei test. There have been two instances of this infection which have come into the Hospital for Tropical Diseases and which had been acquired in this country. There was one typical bubo (the only one I have seen in a woman) in an Irish married woman from Soho and a similar bubo was present in her husband; both gave a positive intradermal test.

Regarding rectal stricture, I am perfectly certain that in the majority of cases it is due to this virus. As a matter of fact, it is very rare in Europeans from the tropics, for out of a series of 3,600 sigmoidoscopic examinations at the Hospital for Tropical Diseases during 16 years I have only got records of two fibrous strictures which gave a positive Frei skin test. I remember the terrible strictures I saw when a student at the London Hospital and how dissatisfied we were at that time with the effect of antisyphilitic treatment upon them.

Dr. Findlay (in reply): Dr. HANSCHELL has suggested that possibly the Frei test is a group reaction. As far as the evidence available goes it appears to be a true allergic reaction comparable to that which is obtained when vaccinia elementary bodies, either dead or alive, are injected into a person immune to vaccinia and present only when immunity has been acquired to the specific virus. My own Frei positive reaction developed some months after I had begun to work with the virus of lymphogranuloma. The development of immunity to virus diseases apart from accidental infection, is now well known in the case of a number of other virus infections such as poliomyelitis and Rift Valley fever. The time after infection at which the Frei test becomes positive is at present unknown nor is it certain whether it is dependent on the continued presence of living virus in the tissues. This question can only be determined by someone who has an opportunity of keeping a patient under observation for a prolonged period.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

COMMUNICATIONS.

ANTIMONY RESISTANCE IN ULCERATIVE GRANULOMA.

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It is generally conceded (MANSON-BAHR, 1935, and RAJAM, 1937) that although antimony, especially the trivalent form, cures ulcerative granuloma in the vast majority of cases, yet there remains a small residue of cases which either relapse after several courses of antimony therapy, or become worse during its administration. Such types of resistance, occurring during the administration of tartar emetic, have been described by LOW and NEWHAM (1917); and by GIGLIOLI (1928), but the last-named observer points out that resistance to tartar emetic does not necessarily imply antimony resistance, as many of his cases, previously resistant to tartar emetic, later reacted favourably to stibenyl. RAJAM (1934) describes three cases which were resistant to urea stibamine, but responded to fouadin, and HANSCHELL (1929) instances a case which, resistant to antimony-potassium tartrate, cleared up when protein shock was combined with that drug.

MAYER and DA ROCHA LIMA (1927) suggest that antimony resistance may be due to mixed infections, which include syphilis, other spirochaetal infection, diphtheria and the Plant-Vincent organism.

In a small series of " antimony resistant " cases observed in Barbados, I was struck by the fact that in each case the Frei test was positive and three of these cases showed such signs of the ravages of lymphogranuloma inguinale as chronic lymphatic enlargement, scars of old inguinal sinuses or oedema of the external genitalia.

It would appear that the resistance of these cases to antimony therapy was due, at any rate in part, to the fact that the granuloma was implanted upon oedematous tissue, a fact that has also been noted by BAYLEY (1937). Moreover, the disease spread with increased rapidity in two cases where oedema was increased by circumcision, and in one case where a lymphogranulomatous oedema of the vulva was further increased by pressure oedema due to pregnancy.

GIGLIOLI (1928) observes that healthy tissue is only with difficulty attacked by the disease: it probably takes advantage of the *locus minoris resistentiae*

*I have to thank Dr. H. H. BAYLEY, of Barbados, for the kind use of his private laboratory for the pathological investigations.

caused by ulcerative or traumatic lesions of the genitals and groins, a theory which is supported by the observations of NAIR and PANDALAI (1934), who have observed the development of the disease in circumcision scars and those following operation on buboes, piles and anal fissures. He mentions two cases and quotes a third by ITURBE of ulcerative granuloma developing in circumcision scars, but unfortunately does not state whether these were associated with genital oedema, showed any marked antimony resistance or had a positive Frei reaction. A case, associated with an elephantoid condition of the vulva, responding to fouadin treatment is described by WILLIAMSON (1933) and co-workers, but the Frei test was not performed. The cases described by RAJAM (1934), which reacted favourably to fouadin, all had negative Frei reactions and included one case of elephantiasis of the labium.

CASE HISTORIES.

The extreme prevalence of venereal disease in Barbados is reflected in this series of cases, which contains no " pure " form of ulcerative granuloma; indeed, one patient had had gonorrhoea, syphilis, ulcus molle and lymphogranuloma inguinale in addition to ulcerative granuloma! In every case lymphogranuloma inguinale either co-existed or had been acquired previously.

The cases were, with the exception of Case 2, of the ulcus molle type described by RAJAM (1937). Case 2 was of the nodular dry type, the lesion being situated on a non-oedematous area, and responding to tartar emetic therapy. Case 3 was a mixed case, the lesion of the ulcus molle type, situated on an oedematous penis, being resistant to tartar emetic; while the dry nodular lesion, situated on the non-oedematous groin, responded completely to tartar emetic.

Case 1.

C. B., a male negro labourer, aged 36, was admitted to hospital on 25th January, 1937. He has a vague recollection of having had a bubo in the right groin many years previously, followed by a slight permanent elephantoid condition of the penis. Five years previously he had had a sore on the prepuce, extending to the glans penis. He was circumcized, the penis became very oedematous, and the sore on the glans continued to increase in size and to erode the penis. The progress of erosion was slow but relentless—the patient attended various out-patient venereal diseases clinics and appears to have had courses of salvarsan and antimony compounds. The first record I could examine showed that on 11.2.36 a course of stibenyl had been given, but no improvement had resulted. Then followed the discovery that his Kahn reaction was weakly positive and he received a course of sulpharsenobenzol; this did not affect the penile lesion.

On admission.—A powerfully built man. Apart from the genital condition, no other lesions could be found. Genital examination showed an almost complete destruction of the penis, only 1·5 cm. remaining. Micturition was free, and there was no dribbling afterwards. Around the penile stump was an almost circular depressed area of pale moist granulomatous tissue, involving the skin of the scrotum. Beyond this, the scrotal skin was undermined by sinuses. The granulomatous area gave a watery purulent discharge having an offensive odour. The scrotum was elephantoid, the testes were normal in size and their sensation was unimpaired. The epididymes were normal. There was a hard, painless enlargement of two of the supero-internal inguinal lymphatic glands of the right side. The prostate and seminal vesicles were normal.

Laboratory Findings.—Wassermann and Kahn reactions negative; Ito-Reenstierna reaction negative; Frei reaction positive. No microfilariae in blood. Scrapings from the lesion showed intracellular capsulated Donovan bodies located inside the large mononuclear and epithelial cells.

Treatment and Prognosis.—As antimony tartrate ointment (1 per cent.) was badly tolerated by the patient—it gave considerable pain—cod liver oil (two parts) and vaseline (one part) was applied to the lesion throughout treatment. From 11.2.37 to 13.3.37 the patient was given a course of tartar emetic injections (6½ grains). Every injection was badly tolerated—the patient always had nausea, often followed by vomiting. He also complained at times of severe pain in the shoulders, pectoral muscles and forearms. The gastric disturbances often lasted several days; this interfered with the regularity of the treatment and accounts for the small total dosage of antimony. During this period (26.2.37) the granulomata were curetted and treated with the electric cautery. The lesion, however, continued to increase in area. From 19.3.37 to 11.4.37 (inclusive) a course of fouadin (35 c.cm.) was given, but this had no effect on the steady increase in size of the lesion. Moreover, a certain degree of joint pain and gastric disturbance followed the administration of fouadin. On 28.4.37, the supply of fouadin being exhausted, a further course of antimony tartrate was commenced, the doses being 2 grains. The lesion continued to spread, and advanced over the os pubis.

Observations.—Severe pain following application of antimony tartrate ointment has been described by NAIR and PANDALAI (1934). It is possible that good results might have followed the use of the steam cautery described by SOUTTAR (1926). Joint pain, headache, nausea and vomiting, following injections of fouadin, have been described by WILLIAMSON (1933), and SÉZARY and BOLGERT (1935) describe similar manifestations of *rheumatisme stibié* with anthiomaline, another trivalent antimony compound.

Case 2.

W. F., a male negro labourer, aged 41, was admitted to hospital on 22nd March, 1937. Several years previously had had gonorrhoea, with peri-urethral abscess, leading to fistula formation, but this had slowly healed up. Five years previously he had a sore on the penis, accompanied by bilateral bubo formation with a fistula on the left side. Salvarsan and bismuth injections were given, the penile lesion healed, the fistula gradually dried up, but the penis and scrotum gradually became elephantoid. Four years ago, whilst working in the Demerara goldfields, a lesion appeared in the angle between the scrotum and right thigh: this gradually increased in size, extending almost to the right anterior superior iliac spine in front, and to the tip of the coccyx behind. Part of the posterior aspect of the scrotum was also involved. The condition was diagnosed as ulcerating granuloma and the patient received injections of tartar emetic, but never had a regular course owing to his work. Remissions occurred, even with partial courses of antimony.

On admission.—A well built man. No lesions found in cardio-vascular, pulmonary, alimentary or central nervous systems. Pityriasis versicolor over chest. Large raised dry nodular ulcerating granuloma in right groin and natal cleft. This gave a thin purulent discharge having an offensive odour. The prepuce was thickened, scarred and non-retractile. The left groin had the scar of a healed sinus. A scar of a healed urethral fistula was seen posterior to the scrotum. Prostate and seminal vesicles normal to palpation.

Laboratory Findings.—Wassermann and Kahn reactions strongly positive; Ito-Reenstierna test positive; Frei test positive. Blood contained no microfilariae. Stools contained no ova. Scrapings from granulomatous tissues showed Donovan bodies.

Treatment and Progress.—From 23.3.37 to 26.4.37 patient received a course of tartar emetic (totalling 23⅔ grains). Rapid healing took place, the new epithelium being in parts white, in parts pigmented. After 3 weeks interval another course was given to prevent relapse, and the patient was ordered to attend monthly for inspection.

Observations.—Here, in non-oedematous tissue, rapid healing took place. The scars were in part pigmented, a fact noted also by GIGLIOLI (1928), although WILLIAMSON (1933) states that the scars always remain de-pigmented.

Case 3.

H. B., a male negro carpenter, aged 21, was admitted to hospital on 25th March, 1937. Three months previously lesions had appeared simultaneously on the prepuce and right groin; these had progressed in size, the penile lesion became painful and the prepuce could not be retracted.

On admission.—A well built subject. No lesions found in cardio-vascular, pulmonary and central nervous systems. Linear granuloma of right groin exuding evil-smelling watery fluid. Penis oedematous. Prepuce non-retractile: pus issued from under it. Scrotum and contents normal. Slight tenderness and enlargement of prostate.

Laboratory Findings.—Wassermann and Kahn reactions negative; Ito-Reenstierna test negative; Frei test positive. Scrapings from both granulomatous areas showed Donovan bodies.

Treatment and Progress.—On 29.3.37 a dorsal slit was performed revealing a granuloma which had considerably eroded the glans penis. A urethritis was present, the pus from which contained gonococci. The rest of the prepuce was removed. From 3.4.37 to 23.4.37 the patient received a course of tartar emetic (15½ grains). On 5.4.37 a bilateral epididymitis developed. At the end of the course of tartar emetic complete healing of the lesion in the groin had occurred, but the erosion of the glans penis had progressed considerably.

Observations.—An example of the disease, in association with a positive Frei reaction and oedema, together with a circumcision wound, progressing during the administration of antimony. Also, in the same subject, ulcerative granuloma, located on a non-oedematous area, which responded to antimony therapy.

Case 4.

J. S., a negress, aged 21, was admitted to hospital on 4th April, 1937. Three years previously had a bubo in each groin: these had later burst, the resulting sinuses slowly healing. Following this the vulva began to swell. Six months previously a sore appeared in the perinaeum and gradually spread to the buttocks, anus and into the vagina. She became pregnant five months ago, and the labia majora, already enlarged, swelled further and gave rise to considerable itching. Coincidentally, the granuloma increased rapidly in extent; became very painful so that coitus had to be abandoned; defaecation also produced great pain.

On admission.—A well-developed girl. No lesions found in cardio-vascular, pulmonary or central nervous systems. Abdominal examination showed a gravid uterus containing a living foetus: the pregnancy was 5 months advanced. Clitoris and labia majora (especially the left) enlarged and elephantoid. In each groin were scars of old fistulae. In the perinaeum was an ulcerating granuloma extending into the anus behind and to the posterior wall of the vagina in front. From it exuded a watery fluid having a foul odour. There was no rectal stricture.

Laboratory Findings.—Wassermann and Kahn reactions negative. Ito-Reenstierna test negative. Frei test positive. No microfilariae in blood-film. Scrapings from granuloma showed typical Donovan organisms.

Progress and Treatment.—From 3.5.37 to 21.5.37 a course of tartar emetic (16 grains) was given. The granuloma continued to spread, and spread throughout a second course of tartar emetic.

Observations.—This is ulcerative granuloma of the ulcus molle type, superimposed upon oedematous tissue produced by a previous lymphogranuloma

inguinale, and further aggravated by pregnancy. Unfortunately, the subsequent history of this case could not be followed up.

Case 5.

Cl. B., a negro porter, aged 26, was admitted to hospital on 18th March, 1937. Two years previously he had had what appeared to be a chancre on the foreskin; his Kahn reaction at this time was strongly positive. He received out-patient treatment (salvarsan), but the penis, which was very oedematous, was gradually destroyed.

On admission.—A well-developed man, healthy except for the genital lesions. The penis was completely absent. Urination was free with some dribbling after the act. At the base of the penis was a granulomatous area (2 inches in diameter) extending over the anterior surface of the scrotum. The scrotum was elephantoid. Small hard lymphatic glands were present in each groin.

Laboratory Findings.—Wassermann and Kahn reactions strongly positive. Frei test positive. No microfilariae in blood-film. Scraping from the granuloma showed intra- and extra-cellular capsulated bodies.

Progress and Treatment.—From 24.3.36 to 21.4.36 a course of neostibosan injections (3 grammes) was given. The granuloma continued to spread. Then followed a course of tartar emetic injections (30 grains), again with no effect on the advance of the lesion.

Observation.—This, again, is a resistant ulcus molle type of lesion, superimposed upon tissue already damaged by lymphogranuloma inguinale.

POSSIBLE CAUSES OF ANTIMONY RESISTANCE.

It is, perhaps, interesting to speculate upon the possible cause of antimony resistance in ulcerative granuloma. It may be due to an antimony resistant strain or to a resistance acquired by exposure to sub-lethal doses of antimony in a manner analogous to that produced in trypanosomes with tryparsamide by YORKE, MURGATROYD and HAWKING (1931). The work of KLEIN (1931) and ALDERSBERG and PERUTZ (1932) has shown that the absorption by artificially produced oedematous tissues of such dyes as congo-red, injected into the blood-stream, is dependent on the chemical constitution of the oedema fluid. Where the oedema fluid is rich in albumin (*i.e.* in wheals produced by injections of histamine or aolan), large amounts of dye are absorbed from the blood-stream. The chemical nature of the oedema fluid produced in the elephantoid conditions following lymphogranuloma inguinale infection may be such as to hinder the absorption of certain of the antimony compounds, so that the causative organisms of ulcerative granuloma are either not reached at all, or in such sub-lethal doses as to produce acquired resistance to antimony. Protein shock therapy, in conjunction with antimony treatment, may lead to a histamine or lymphogogue action in the oedematous area, with increased albumin-content of the fluid and, in consequence, absorption of lethal doses of the antimony compound.

SUMMARY.

1. Five cases of ulcerative granuloma, of which four were resistant to antimony therapy, are described.

2. The association of these resistant cases with previous lymphogranuloma inguinale infection and oedema of the infected area is pointed out.

3. A tentative explanation of the production of antimony resistance in oedematous tissues is submitted.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

SOME NOTES ON SCORPION POISONING IN TRINIDAD.

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INTRODUCTION.

The scorpions belong to the Phylum Arthropoda; Class: Arachnida, Order: Scorpionidea, and are grouped into seven families :—

1. Buthidae. 2. Scorpionidae. 3. Ischnuridae. 4. Chaerilidae.
5. Chactidae. 6. Vejovidae. 7. Bothriuridae.

They are found in all warm parts of the world, and are nocturnal and very rapacious, feeding on spiders and insects. Human beings are only stung accidentally.

When the scorpion is interfered with it brings into action its defensive and offensive apparatus. The sting is situated in the last segment or "telson" which is globular in shape and ends in a long curved spine, near the apex of which the ducts of the poison glands open. The pair of poison glands are situated in the globular part and are separated by a muscular septum, the contraction of which forces out the poison. The scorpion never stings backwards but always in front of itself; it consists of a broader front portion—the cephalo thorax and abdomen, and a narrower one, the tail.

"The venom," according to JACKSON (1910) and KUBOTA (1903), "is a transparent liquid; when agitated it produces a froth; it is acid in reaction; when evaporated, it leaves scaly flakes of dark yellow colour, which are soluble in water, normal saline, glycerine, and dilute alcohol. Pure alcohol, iodine, ether, ammonia and tannin precipitate the poison. Heating to 100° C. for 30 minutes destroys the poison. The toxic principle is in the nature of a tox-albumin, a neurotoxin, which resembles cobra venom and acts on the medulla and motor and plates causing death by paralysis of the respiratory system."

* I have to thank the Director of Medical Services, Trinidad, for permission to publish these notes, also Drs. PAWAN and GOSDEN for Laboratory investigations and Drs. ROSTANT, BOUCAUD and TOI A SUE for informing me of some of their cases.

It also has a lecithide which haemolyses nucleated as well as non-nucleated blood corpuscles, as do the lecithides of cobra venom. (KYES, 1903). Although the sting of a scorpion is very painful the poison as a rule does not produce general symptoms in adults, but in children under 5 years of age the sting frequently causes death.

According to STITT (1923) the mortality among young children stung by *Buthus quinquestriatus* is 50 per cent.

BYAM and ARCHIBALD (1923) quote Dr. MOHAMMED SHAHEEN who states that the mortality under 5 years of age is 60 per cent. In Trinidad the case mortality rate under 5 years is 25 per cent.

The toxicity of scorpion venom varies in different countries and even in different sections of the same country. WILSON in 1904 recorded many deaths in the Sudan.

The Durango scorpion of Mexico—*Centruroides suffusus*—was responsible for 1,608 deaths between 1890-1926 in the city of Durango with a population of 40,000 (BAERG, 1929).

In Southern Europe and North Africa the black scorpion, *Euscorpius italicus*, causes many fatalities. The high death rate led TODD (1909), of Cairo, to prepare an antivenine which is given in doses of 10 c.c. for adults, and 5 c.c. for children. It is prepared by immunizing horses against scorpion venom and using the serum. In Manchuria, *Buthus martensi* is the most venomous. In Trinidad the black scorpion is the most venomous and produces in addition to the nervous and haemolytic symptoms usually described, hyperglycaemia, glycosuria, pancreatitis, pancreatic cysts and cardiac irregularities.

The virulence of the venom varies with the seasons—during the hot and breeding season it is more toxic.

The etiology of acute pancreatitis will have to be revised in view of its occurrence following scorpion sting, as it is reasonable to assume that other toxins can affect the pancreas. The prevalence of diabetes mellitus may be due to toxins of acute infectious diseases partially damaging the pancreas, which has to carry on its work under great strain and sooner or later breaks down, thus producing diabetes.

SCORPION POISONING IN TRINIDAD.

1.—*Incidence.*

It is very difficult to gauge accurately the incidence of poisoning by scorpion stings, as only the more severe cases consult a doctor or gravitate to a hospital. In certain districts of the Colony scorpions are more numerous, and perhaps more venomous. The cane fields of southern Trinidad from Couva to Siparia and the cocoa plantations of the north-eastern part account for the majority of the fatalities. In the coconut plantations the scorpion is not so numerous or its sting is apparently less dangerous. It is commonly found among the clothing and in shoes. Its presence in the home is not an index of uncleanliness.

Fowls, by eating the young scorpions, may be considered their natural enemies.

2.—*Age and Sex.*

Sex has some influence in the incidence of scorpion stings for we have more males working in the plantations and so exposed to the dangers of stings. The number of cases increases *pari passu* as the age for the same reason.

Fewest cases occur in infancy and childhood and the majority from 13 years of age upwards.

3.—MORTALITY RATE AND INCIDENCE.

The mortality rate for the whole Colony is unobtainable, but the following figures have been derived from the records from 1929-1933 of one of the hospitals situated in the neighbourhood of a cane-growing district.

Year.	Number of Cases.	Number of Deaths.	Case Mortality per cent.
1929	136	7	5·14
1930	135	6	4·4
1931	126	4	3·17
1932	112	3	2·67
1933	189	13	6·87
Totals	Cases : 698	Deaths : 33	Average mortality : 4·7 per cent.

Mortality according to Age and Age Groups.

Age.	Cases.	Deaths.	Case Mortality per cent.
Up to 1 year	16	6	37·5
Over 1-2 years	15	3	20·0
„ 2-3 „	20	9	45·0
„ 3-4 „	18	2	11·0
„ 4-5 „	19	2	10·5
Age Group.			
1-5 years	88	22	25·0
6-10 „	96	5	5·2
11-20 „	190	5	2·6
Over 21 years	324	1	0·25

Seasonal Distribution of Cases and Mortality.

Month.	1929.	1930.	1931.	1932.	1933.	Total.	No. of Deaths.
January	13	5	4	10	18	50	—
February	8	4	7	9	6	34	—
March	6	3	7	5	11	32	2
April	3	9	8	12	12	44	1
May	6	12	8	19	10	55	1
June	30	21	12	10	25	98	9
July	20	18	21	9	24	92	5
August	16	15	18	13	15	77	4
September	9	17	21	9	22	78	4
October	5	14	13	7	21	60	2
November	12	11	6	3	14	46	2
December	8	6	1	6	11	32	3

Age of patients who died.

One each at 5 months, 8 months, 1 year and 3 months, and $2\frac{1}{2}$ years.
Two at 2 years, four at 1 year, eight at 3 years, two at 4 years, two at 5 years, two at 6 years, two at 7 years, two at 15 years, and one each at 9, 12, 14, 18, and 45 years.

The high death rate among children under 5 years of age is to be expected for the infant receives the same amount of poison as an adult, and as the volume of blood is much less the concentration of the poison will be much greater. Secondly, an infant has not the same resistance as the adult to disease, and thirdly, its nervous system is not yet fully matured as is frequently demonstrated by the development of convulsions in numerous conditions : teething, worms, etc.

Morbid Anatomy.

All the organs, especially the stomach, brain, kidneys, liver and heart, show congestion, certain organs show in addition other lesions as if they had borne the brunt of the infection, *viz.*: lungs in 25 per cent of the cases show gross oedema, probably due to heart failure ; small haemorrhages under the visceral pleura were sometimes seen, and also submucous haemorrhages in the stomach and subcapsular haemorrhages in the kidneys. The brain meninges were generally congested, with haemorrhages occasionally. The heart showed an increase in pericardial fluid and occasionally subendothelial haemorrhages. The pancreas showed the following appearance in ten cases in which it was examined : a normal appearance in four, congested and enlarged in two, congested with haemorrhages in two, and acute haemorrhagic pancreatitis in two. The blood on one occasion appeared rose red.

The virulence of the toxin can be gauged by the celerity with which it kills its victims. In twenty cases the intervals between the sting and death

were as follows (in hours): $1\frac{1}{2}$; 2; $4\frac{1}{2}$; 5; 6; 7; $7\frac{1}{4}$; two at $7\frac{1}{2}$; 12; two at $12\frac{1}{2}$; 15; $15\frac{1}{4}$; $15\frac{1}{2}$; $16\frac{1}{2}$; two at 19; $21\frac{1}{2}$, and 42 hours.

Symptomatology.

Soon after the patient has been stung he experiences a very sharp burning pain at the site of the puncture, this pain generally passes off within 15 minutes but occasionally it persists for hours and local treatment has to be applied. Sometimes the area around the site becomes slightly swollen and the site of the puncture can be made out. After a variable interval of $\frac{1}{4}$ hour to 2 hours the patient begins to feel sick and if at work is unable to continue. Salivation followed by nausea and vomiting which may be very persistent then occur. The pulse is generally slow and the temperature subnormal, the respirations are fast. The urine usually contains sugar and at times albumin. The vomiting may continue accompanied by profuse perspiration, and the patient may die of heart or respiratory failure.

The patient may have an anxious facies or appear excited or very frightened: when one considers that death may occur $1\frac{1}{2}$ hours after being stung—a fact known to many—it is no wonder that the feeling of impending dissolution should show itself in fear.

The effect of the poison on the different systems will now be dealt with in greater detail, after which some typical cases will be described.

Alimentary System.

This system seems to be the one attacked very early and often severely. Salivation is always present in varying degree, accompanied with nausea: it may be the only symptom. Vomiting is practically always present and may commence $\frac{1}{4}$ hour after the patient has been stung. At first, the stomach contents are ejected then bilious fluid in seemingly endless quantities is brought up, finally the vomiting ceases and the patient gradually recovers, but in some cases the vomiting persists and the vomitus appears as "coffee grounds" or pure blood. This in itself does not connote a bad prognosis. In only one case has diarrhoea accompanied the vomiting. A frequent and early symptom is tenderness and actual pain in the epigastric and umbilical regions; at times the abdomen is distended and rigid with generalized pain and tenderness suggesting an acute abdomen. Although the persistent vomiting and retching, which continues for some time after the actual vomiting ceases, may to some extent account for the epigastric pain and tenderness, yet it seems likely, in view of the lesions frequently found in the pancreas, that the symptoms may be due in part to inflammation and congestion of that organ.

Circulatory System.

In the adult, the heart beat is generally slow but forcible. Extra systoles are comparatively frequent and occasionally the pulse is irregular in force and

rhythm: often the pulse rate is 48 per minute. Frequently the respirations approximate the pulse, *e.g.*, in one case temperature was 97° F.; pulse 48; respirations 48. In children the tendency is for the pulse to be fast or normal, especially in severe cases with cold sweats and collapse, but even here the heart may be slow as the following taken from notes of three cases: T. 97°, P. 72, R. 30; T. 97°, P. 68, R. 56; T. 97°, P. 72, R. 36.

Blood Pressure.—The systolic pressure is generally above and the diastolic below normal. Frequently the pulse pressure is equal to and at times greater than the diastolic pressure. The pulse reminds one of Corrigan's pulse.

The following blood pressures taken from different patients may be given as illustrations :—

	Blood Pressure.		*Blood Pressure.*
S., age 12 years.	116/55 (2.10.34).	R., age 14 years.	150/80.
	115/60 (3.10.34).	S., ,, 20 ,,	115/65.
	105/60 (4.10.34).	B., ,, 21 ,,	170/80.

The Respiratory System.

The respirations are always increased. The normal pulse respiration ratio of 4 : 1 becomes 3 : 1 or 2 : 1 and at times 1 : 1. Frequently the breathing is laboured and there is obvious dyspnoea but there is never cyanosis. The alae nasi are actively moving as though the patient cannot obtain sufficient air. A tentative explanation will be put forward during the discussion on the pathology.

The temperature in the adult may be subnormal at first, but soon becomes normal and remains so. Occasionally there may be a rise to 99° or 100°. In the case of infants and children although they may be admitted in a collapsed condition the temperature soon rises to 101° but, in spite of the apparent reaction from the stage of shock, sudden deaths in this stage are not uncommon. During convulsions the temperature may rise to 105° or 107° F. and yet the patient may recover.

The Nervous System.

There seems to be at times a general irritability or hyper-excitability of the nervous system. The abdominal reflex may be very active, the knee jerks exaggerated. Knee and ankle clonus often obtained but not well sustained. Babinski is positive only on the side of the sting. Hiccough was noted in a few cases. Headache is a frequent symptom and sometimes persists for a day or two after the patient is apparently well. Epigastric and umbilical pain and tenderness have been mentioned already. Tremors are occasionally noted. Convulsions are common in infants and children and when they occur the prognosis becomes much worse but not hopeless. In adults convulsions have not the same serious significance but occasionally an adult succumbs. The convulsions may resemble those of eclampsia with a short tonic stage and a longer clonic stage.

Frequently after a severe case of the gastric type or following convulsions the patient becomes drowsy and this lethargic state may last for days. Coma is a frequent sequel especially if convulsions have been present. At times the patient becomes delirious. Temporary hemiplegia or monoplegia may occur. Sometimes the patient may complain of darkness of vision and a case where a patient became absolutely blind for a short time has been described by INNISS (1927). Incoordination of upper and lower extremities is met with. The following symptoms, "the tongue is sluggish, so that communication is often impossible to give medicine through the mouth," described by SANTA MARIA, by signs. The muscles of the lower jaw are contracted so that it is difficult or (1893) by JACKSON (1910) and others as being typical of the Durango scorpion, are not met with in Trinidad; on the contrary, the patients cry out with thirst and hunger and consume large quantities of fluid.

Urine.

Urine is generally transparent, acid in reaction, no sediment, specific gravity 1015–1035. Sugar in large amounts is present in 50 per cent. of cases. In one case 3·6 per cent. $3\frac{1}{2}$ hours after the sting. Diacetic acid and acetone are present occasionally, a trace of albumin is generally present with large amounts occasionally. Bile is absent.

Blood.

Blood sugar examinations reveal a hyperglycaemia which readily disappears without insulin.

Complications and Sequelae.

1. Pancreatic cysts have been found in twelve cases at varying intervals following scorpion stings.
2. Persistent mental deterioration in one patient has been recorded.
3. Persistent cardiac irregularities, *e.g.*, extra systoles.

Diagnosis.

The diagnosis is generally easy if a history of the sting is obtained, a slow full pulse easily compressible, with rapid respirations, a pulse respiration ratio of 3 : 1, 2 : 1, or 1 : 1; salivation, vomiting, glycosuria and epigastric pain and tenderness is a characteristic picture of scorpion sting.

In infancy and childhood the diagnosis is more difficult because often no history of scorpion sting is obtained, simply that the child is suffering from convulsions. In these cases MATTHEW DUNCAN'S trite saying is of importance, namely, "if we do not know a condition we will never suspect it and if we never suspect it we will never find it." Even in these cases salivation,

vomiting, sugar in the urine and perhaps the pulse respiration ratio will be sufficient to diagnose the case. If the child is collapsed and not vomiting but rather drowsy, then inquire whether the child vomited or salivated much at home. Another important question to ask is whether the child gave a sudden cry sometime before becoming ill. If still in doubt then malignant malaria must be excluded, also acute infectious diseases, etc.

The differential diagnosis in a case of a pregnant woman stung by a scorpion and developing convulsions from one suffering from eclampsia is very difficult unless much care is given to the case. The raised blood pressure, the epigastric tenderness, headache, dimness of vision and convulsions are common to each. The pulse respiration ratio and the presence of glucose in the urine should be a sufficient guide to a correct diagnosis.

The effect of Scorpion Poisoning on Pregnancy.

Four cases, 6, 6, 7 and 8 months pregnant respectively, were treated for scorpion sting without any effect on their pregnancies, but one case, 8 months pregnant, developed convulsions and was delivered 2 days afterwards of a healthy infant.

The Excretion of Scorpion Venom through the Mammary Gland.

One of my confrères narrated the following interesting case: the mother was stung by a scorpion with little effect shortly before nursing her child, which then showed symptoms of scorpion poisoning and died.

FRANCIS and FAYRER (1868) have recorded a case in which snake venom passed through the mammary gland. "A poor Mussulman woman died at Madras from the bite of a cobra, she was nursing her child at the time and the latter succumbed in its turn a few hours later with all the symptoms of poisoning, although it had not itself been bitten and had been suckled by its mother only once since the bite."

Prognosis.

Prognosis varies with the age of the patient, it is very bad in children under 3 years of age and improves with increase in age. The virulence of the different types of scorpion is being worked out at present. Convulsions and unconsciousness are of evil omen especially in children. Severe shock with profuse cold sweats, fast thready pulse, subnormal temperature, is bad. The prognosis should always be guarded in children, for too often cases recover from the initial severe shock and appear much improved, and then suddenly die of heart failure. As the prognosis primarily depends on the amount of toxin injected into its victim by the scorpion, it necessarily follows that the size of the scorpion, the contents of its sac at the time of stinging, and whether the puncture was

made directly through the skin or whether through clothing, must be taken into consideration.

The time of the year seems to have some effect on the death rate. During the months June to September there were 345 cases with a case mortality of 6·4 per cent. and for the other 8 months 353 cases with a case mortality of 3·1 per cent.

The site of the sting appears to have no influence on the prognosis. The time that elapses after being stung and the onset of the symptoms would seem to have no prognostic importance. The sooner treatment is started after the sting the better the prognosis.

Pathology.

It is very intriguing to consider that this symptom complex—consisting of (1) The slowing of the heart, (2) Rapid respirations, (3) The hyperglycaemia and glycosuria, (4) The salivation and vomiting, (5) The vasomotor disturbance—is due to the action of the venom on the fourth ventricle, where the different centres are situated.

The morbid anatomy and clinical findings will not permit of such an easy explanation. The hyperglycaemia may be due to stimulation of the sympathetics which acting on the liver converts glycogen into glucose as occurs in Claud Bernard fourth ventricle puncture experiment. In as much as the pancreas is found sometimes to be in a state of haemorrhagic degeneration, we are forced to conclude—until further investigation is carried out—that the supply of insulin may be temporarily interfered with and therefore the glucose in the blood cannot be metabolised. No doubt the conversion of liver glycogen into glucose plays an important part in the production of the hyperglycaemia and glycosuria, for the following reasons :—

1. The sudden onset of severe glycosuria.
2. The equally sudden disappearance of sugar from the urine at times.
3. The appearance and disappearance of the hyperglycaemia is nearly as sudden.

The irregular actions of the heart such as extra systoles, partial heart block, appears to be more likely due to the action of the toxin on the conducting fibres in the heart wall, than to the toxin acting centrally on the vagus.

The salivation and vomiting are no doubt due in part at any rate to the excretion of the toxin by the salivary glands and mucous membrane of the stomach. The submucous haemorrhages and the vomiting of " coffee grounds " material and often pure blood reveal the haemolytic nature of the toxin.

The albuminuria is the result of the toxin on the kidney parenchyma.

The increased respiratory rate was one of the most fascinating features in some of these cases and could not fail to attract the attention of the clinician.

In the absence of a complete biochemical examination of the blood it is difficult to be dogmatic as to etiology but the following observations and deductions are stated for what they are worth.

In some cases acetone and diacetic acid were found in the urine. One cannot help coming to the conclusion that an acidosis develops as in diabetes mellitus.

The explanation given in text-books is, that because some of the " alkaline bases " have to be diverted in order to keep pH concentration of the blood fairly constant, the remainder of these bases in order to remove the CO_2 in the tissues and bring O_2 to them, must make more trips to the lungs, which work at an increased rate to meet the demand.

No doubt the slow pulse rate intensifies and accelerates the action of the lungs and may be physiological. Many of the signs and symptoms occurring in these cases remind one of the condition of acute hypoglycaemia which sometimes occurs after a large injection of insulin. It is true that there is a large amount of sugar suddenly discharged into the blood stream, producing a hyperglycaemia, but, this sugar may be considered to be " raw " and not the same as the " refined " sugar which is normally and slowly manufactured from glycogen. Or to put it more scientifically, the molecular constitution of the glucose which we may call B glucose circulating in the blood and produced rapidly by the action of the scorpion toxin, is quite different from the molecular constitution of the glucose normally produced by the body and which we call A glucose.

Now B glucose cannot be metabolized by the tissues and therefore is excreted in the urine. The tissues of the body are thereby deprived of their sugar supply with the result that symptoms of acute hypoglycaemia follow: namely, signs of shock, pains in the epigastrium, rapid respirations, convulsions, coma and death.

The production of shock varies in time of onset and severity: we may explain the onset of shock as due partly to the relative hypoglycaemia and partly to the action of the toxin on the nervous system, especially the vasomotor system. In adults, suffering from scorpion poisoning, we find that the pulse is slow, the cardiac impulses strong, the diastolic pressure which depends on the integrity of the vasomotor system is very low, with the result that the pulse pressure is high, at times even greater than the diastolic pressure. It seems as though the slowing of the heart is Nature's method of conserving her supply of glucose available to the heart and of keeping the circulation going in spite of the dilatation of the veins and capillaries. It is only when this fails as it frequently does in children, no doubt because their heart muscle cannot accommodate itself or stand the necessary strain, that we get signs of severe shock.

The signs and symptoms of acute abdomen met with in some cases are no doubt due to acute pancreatitis, the result of the toxin acting on the pancreas. The diastatic index of the urine may be 167 as stated in one case.

TREATMENT.

Prophylactic.

It would appear from the fact that many persons pass through several attacks of acute scorpion poisoning that no immunity can be acquired. The following histories illustrate the point :—

1. Stung three times before but never vomited as much as on this occasion.
2. Stung 4 years ago and had to be treated in hospital and again this year and had to be brought to the hospital for treatment.
3. Stung ten times before, was in hospital a year ago and had to be brought again this year.
4. Stung several times before and suffered no ill effects, but on this occasion ½ hour after being stung felt nauseated and soon afterwards began to vomit.
5. Stung by a scorpion and had to be treated at hospital, was stung again 2 days afterwards and had to be re-admitted.

Nevertheless, the inhabitants have great faith in their methods of producing immunity :—(*a*) Fry a few scorpions and eat them. (*b*) Smoke the scorpion in a pipe. (*c*) Place some scorpions in rum for a week or two and then drink the contents.

A method practised in British Guiana is worthy of note as it is a form of vaccination, the " telson " of the scorpion is used as a needle to scarify a small area of the skin and then a small amount of the poison is rubbed on the raw surface.

Serological.

An antiserum has been prepared in some countries but its action is specific to the particular scorpion. CALMETTE'S (1908) announcement that the toxic principles of all different origins including snakes and scorpions are the same, suggested the use of his anticobra serum as a remedy for scorpion poisoning, but this proved a failure.

Local.

(*a*) Apply a tourniquet immediately over a single bone some distance above the site of the sting, then make an incision at the site and apply potassium permanganate to the wound or apply the cautery.

(*b*) Another line of treatment is to place a tourniquet just above the site of the puncture and every ½ hour apply another tourniquet higher up the limb and remove the lower one. This method aims at : (*1*) allowing only a small amount of toxin to enter the circulation at any one time ; (*2*) keeping the

toxin n contact with the tissue fluids which may " fix " the toxin or render it innocuous. The difficulty with these methods is that by the time the patient reaches the doctor the toxin is already in the general circulation.

If the pain at the site of the sting is severe an injection of novocaine relieves it quickly.

Medical.

Many drugs have been used. One drug, potassium permanganate, was used extensively intravenously in scorpion sting cases, but it has been discontinued. It is used locally for its oxidizing power as in snake bite. It was recommended by Professor DE LACERDA (1881).

More recently 2 c.c. colloidal manganese 0·25 per cent. have been injected intramuscularly and may be repeated in an hour's time if necessary. INNISS (1927), who has much faith in this drug, believes that the drug acts through its manganese content.

Now that the pathology is understood a little better a new line of treatment is being adopted. Glucose 10 per cent. in saline is given rectally, or a 3 per cent. may be given subcutaneously, followed by an injection of insulin which may be repeated if necessary. Blood sugar estimations are carried out whenever possible. Shock is treated in the usual way by warmth, pituitrin, and saline administration. Adrenalin is supposed to check vomiting. When convulsions occur morphia should be injected in large doses even in children. Chloroform is the remedy *par excellence* according to H. V. JACKSON (1910). A few inhalations would render the patient quiet and usually put him to sleep.

Sedatives recommended are ammonium bromide, chloral hydrate and hyocine.

SUMMARY.

Figures showing the incidence of scorpion poisoning and case mortality are presented.

The appearance of glycosuria, hyperglycaemia, acute pancreatitis, pancreatic cysts, and cardiac irregularities are described and typical cases given.

The diagnosis of acute pancreatitis is often missed, because, in the first place it is not easy to diagnose, secondly the acute abdominal pain may be attributed to the vomiting and retching.

Pancreatic cysts appear with dramatic suddenness on some occasions.

It it hoped this paper will stimulate other workers on scorpion sting to look out for the features mentioned above, as they are not found in the literature I have been able to obtain.

CASES.

Case 1.—*S. M., age* 50.

Brought to my office on 21.1.25 with a history of having been stung by a scorpion on the previous day, and of having convulsions. The heart beat was fast and irregular in force and rhythm. The lungs showed signs of chronic bronchitis; 1/100 grain digitalin was injected and soon afterwards he had a convulsion in my surgery. He was sent to hospital, where convulsions continued at intervals until 23.1.25 when he died.

From this incident I regarded scorpion poisoning as a serious condition and, no doubt, it caused me to take an interest in the subject.

Case 2.—*Boy aged* 6 *years. Convulsive type causing mental deterioration.*

In 1928 was seen at 11 p.m. with the history of having been stung by a scorpion at 5 p.m. that afternoon. He had vomited several times at home and then started to get convulsions. He had several typical eclamptic convulsions. After several injections of morphia and not before 2 a.m. was I able to retire to bed with an easy conscience but a heavy heart. The next day he was very drowsy and dull, this I attributed to the injections of morphia, but days passed and his mental dullness persisted. Three months afterwards the father reported to me that his boy previously bright was now dull and stupid.

Case 3.—*J. F., age* 8 *years, schoolgirl. Transient hemiplegia following convulsions due to scorpion sting.*

Admitted to hospital 8.30 p.m. on 26.8.34 with the following history of having been stung on her left shoulder at 7.30 p.m. by a scorpion, and had vomited several times.

On admission T. 98·4°, P. 76, R. 28, she was conscious and was still vomiting bilious fluid.

Heart and lungs: nil abnormal. Spleen and liver: not enlarged. Urine: sugar ++, albumin nil.

27.8.34. 2 a.m., T. 101°, P. 100, R. 36, and at 2 a.m. she started to have convulsions, they were generalized and too numerous to count. At 6 a.m.: T. 101·4°, P. 96, R. 38. 10 a.m., T. 102·6°, P. 104, R. 32. She was now in a semi-comatose condition with conjugate deviation of her eyes to the right. 2 p.m., T. 99·4°, P. 96, R. 28. 6 p.m., T. 99°, P. 100, R. 28.

28.8.34. 2 a.m., T. 98·4°, P. 88, R. 20. At 6.30 a.m., flaccid paralysis of the right arm and right leg was noticed. Urine: sugar and albumin, nil.

29.8.34. 8 a.m., some power returning to the paralyzed limbs and the leg was recovering much faster than the arm. It was noticed that the right plantar reflex was extensor.

30.8.34. She was able to walk about, but right upper limb showed marked incoordination: the patient was discharged well 3.9.34.

The routine treatment as stated in the body of this paper was adopted.

Case 4.—*H. H., male, aged* 40. *Convulsions and blindness the result of a scorpion sting.* (This case was reported by INNISS, 1927).

The man was stung on the right leg at 11 a.m., while at work in the field, by a scorpion, which he saw and killed. Seen at 2 p.m., vomiting, restless, and feeling very ill. The pulse was 114, respiration 70, deep and full. Urine: sugar + and albumin ++. Colloidal manganese 2 c.c. was injected and his pulse, in 15 minutes, was 100 and respirations 54. 2.35 p.m., patient complained of blindness, ¼ grain morphine hydrochloride was injected. 2.40 p.m., patient had a typical eclamptic convulsion lasting 2 minutes. He recovered consciousness, and ¼ grain morphia and 2 c.c. colloidal manganese were injected. 3 p.m., had another very violent convulsion, remained deeply unconscious for 20 minutes. After

the fit the pulse became small and rapid and the respirations nearly stopped. 7 mns. adrenalin chloride 1-1,000 and 1½ c.c. manganese were injected. The prognosis appeared very gloomy. 3.30 p.m., consciousness increasing, injected ¼ grain morphia. 4.20 p.m., fully conscious and intelligent. Pulse regular and strong. He slept that night. Next morning pulse 76, respirations 21.

For several days he suffered from headache and insomnia, but eventually recovered.

Cases Developing Pancreatic Cysts or Pseudo-pancreatic Cysts following Scorpion Stings.

Case 5.—*R. B., age* 13, *schoolgirl. Developed a pancreatic cyst after being stung by a scorpion.*

Admitted 6.6.34 with a history of having been stung by a scorpion on the right big toe at 2 p.m. on 5.6.34. The scorpion was killed by her mother and was carrying young ones on its back. Three-quarters of an hour after being stung she began to vomit and had pain in her epigastrium.

On admission T. 98°, P. 68, R. 20. Vomiting in ward and complaining of epigastric pain. Urine : sugar ++, albumin nil.

7.6.34. 2 p.m., T. 100·4°, P. 104, R. 28. No vomiting but pain still in epigastrium. Urine : sugar and albumin nil. 6 p.m., T. 101°, P. 120, R. 28.

8.6.34. Fairly comfortable. 6 a.m., T. 99·8°, P. 100, R. 28. 6 p.m., T. 99·6°, P. 96, R. 32.

9.6.34. 6 a.m., T. 98°, P. 76, R. 20. Discharged.

Re-admitted on 24.6.34 with a history that on 16.6.34 she began to suffer from pains in the epigastrium and back opposite stomach, and 4 days afterwards she noticed a swelling in her epigastrium. Urine : sugar and albumin nil.

On admission, 24.6.34, T. 98·4°, P. 100, R. 20. A tense smooth tender swelling in epigastrium and extending from umbilicus to the xiphisternum.

27.6.34. Tumour smaller and pain less.

2.7.34. Tumour practically disappeared. Discharged.

Re-admitted on 13.8.34 with a history that the swelling returned a week ago. On admission, urine : sugar and albumin nil. Cystic tumour in epigastrium.

15.8.34. Urine albumin nil.

11.9.34. Operation. Left paramedian incision. Pancreatic cyst revealed. Cyst incised and tube inserted. Small area of omentum showed signs of gelatinous degeneration.

12.9.34. Fluid from cyst was dark brown in colour and contained large quantities of albumin, no sugar, diastase present.

27.9.34. Tube removed.

1.10.34. Discharged.

Case 5 (*a*).—*V. P., age* 7, *schoolgirl. Developed a pancreatic cyst a few weeks after being stung by a scorpion.*

Admitted 6.5.33 with a history of having been stung by a scorpion about 3 weeks ago, and 2 days ago her father noticed a swelling in her abdomen. She suffers from intermittent pains in her abdomen and her mouth fills with saliva frequently.

On admission, T. 98°, P. 126. Heart and lungs : normal. Abdomen : tense rounded smooth fluctuating swelling in epigastric region, extending from umbilicus to xiphisternum. Patient was very anaemic. Urine : nil abnormal.

8.5.33. Tumour appeared larger. Peristalsis waves could be seen passing from left to right. Complained of intermittent pains.

Blood examination : Hb. = 67 per cent ; Lymphocytes = 14 per cent. ; Eosinophil = 10 per cent. ; Large mononuclears = 9 per cent. Stools examined : no ova were found.

23.5.33. Operation. Left paramedian incision above umbilicus. Large cyst presented itself as though springing from the pancreas. The peritoneum was shut off, an incision made in the cyst wall and large tube inserted. Abdomen closed.

24.5.33. T. 97°, P. 102. No vomiting, complaining of abdominal pain. Examination of cyst fluid : albumin +++, sugar +, cholesterin, red and white cells.

16.7.33. Discharged well.

Case 5 (*b*).—*H. L., age* 20.

History : Stung by a scorpion in June, 1936.

On admission there was a large cystic swelling in the epigastric area.

6.10.36. Operation revealed a large unilocular smooth walled cyst. Marsupialization performed.

Examination of fluid : (1) Reaction alkaline ; (2) protein present ; (3) chlorides present ; (4) a trace of urea ; (5) diastase present ; (6) no trypsin ferment.

Case 5 (*c*).—*R.*

Stung by a scorpion in January, 1936.

4.4.36. Operated on for a cyst in the epigastric region.

Examination of fluid : (1) Reaction neutral ; (2) large quantities of proteins ; (3) diastase present ; (4) no sugar ; (5) no organisms on smear or culture.

Case 5 (*d*).—*S., age* 15 *years, male.*

Admitted 10.25 a.m., 23.10.37. History : Stung by a black scorpion on 23.9.37 ; about a week afterwards a swelling appeared in his abdomen. This swelling has been increasing in size and causing much pain.

On admission there was a large cystic swelling occupying the epigastric region, size 5½ in. × 7½ in.

26.10.37. Ether and oxygen. Marsupialization of pancreatic cyst performed.

Cyst Fluid.

Quantity : 2 pints.
Colour : brown.
Reaction : alkaline.
Proteins : +.
Chlorides : +.
Trypsin present.
Tryptic activity — 1.
Diastase present.

Microscopic Examination.

Epithelial cells. Red cells. Fibrin threads.

Blood.

Red cells : 4,500,000.
White cells : 8,918.
Hb. : 75·9 per cent.
Neutrophils : 80 per cent.
Eosinophils : 4 „
Small lymphocytes : 8 per cent.
Large „ 4 „
Mast cells : 1 per cent.
Large hyaline : 3 per cent.

Sugar Tolerance.

Specimen : Blood : 0·070 per cent. ; Urine : no sugar.
50 grammes glucose given by mouth :
¾ hour after : 0·154 per cent. ; no sugar.
2 hours after : 0·076 per cent. ; a trace of sugar.

Diastatic index : 167 units.

CASES WITH NOTES ON THE EXAMINATION OF BLOOD AND URINE.

Case 6.—*P. F., age* 21.

Admitted 14.9.34 at 11.10 a.m. with a history of having been stung by a scorpion on her left shoulder at 8 a.m. Vomited several times greenish fluid at home. Had been stung three times previously but never suffered as much as on this occasion.

On admission, T. 98°, P. 60, R. 48. Heart and lungs : nil. Urine : sugar +++, albumin nil. Salivating and vomiting in ward. 6 p.m., T. 98°, P. 72, R. 32.

Laboratory report :—Blood : 12.30 p.m., sugar 0·26 per cent.
„ 12.55 p.m., „ 0·24 „
„ 1.50 p.m., „ 0·19 „

Urine : 11.45 a.m., „ 2·0 „
„ 12.30 p.m., „ 1·7 „

15.9.34. T. 98°, P. 72, R. 32. Urine : no sugar or albumin present. Discharged.

Case 6 (*a*).—*L., age* 15, *male.*

Admitted 1.55 p.m., 18.9.34. History : stung while working on a cane field at 11 a.m. on the fourth toe of left foot, and began to vomit at noon. Was stung 2 months previously but only suffered from salivation.

On admission : T. 98°, P. 68, R. 24. Pulse full and regular. Heart : forcible beats Lungs : nil abnormal. Alae nasi moving rapidly. Complaining of pains in epigastrium. Salivating profusely.

Urine examination :—2.30 p.m., sugar 3·6 per cent., no diacetic acid, no acetone.
7.0 p.m., „ 3·6 „ „ „
9.0 p.m., „ 1·6 „ „ „
12 midn't, „ nil, doubtful „ „
19.9.34. 6.0 a.m., „ nil, diacetic acid present, trace of acetone.

Case 6 (*b*).—*E. P., age* 9, *schoolgirl.*

Admitted 6.30 a.m., 24.2.37. History : Stung by a scorpion on her left arm and shoulder and right thumb at 4.50 a.m., 24.2.37. She vomited twice on her way to hospital, complained of pains in epigastrium. On admission : T. 97·2°, P. 92, R. 72. Heart : very irregular, dropped beats and extra systoles. Lungs : nil. Breathing very fast. Alimentary system : *salivating, epigastrium very tender, also liver which was slightly enlarged. Spleen :* enlarged. Central nervous system : nil abnormal to note. Headache very troublesome. Blood pressure : 140/75. Vomited several times.

Between 12.30 p.m. and 4.25 p.m. she had seventeen convulsions. Nature of convulsions :—

1. She regained consciousness soon after fit was over.
2. They were generalized, starting frequently in the left upper limb.
3. Duration varied from ½ minute to 4 minutes.
4. She did not bite her tongue or foam at the mouth.

Treatment.—Four injections of morphia 1/8 grain each ; potassium bromide and chloral ; glucose rectally and per mouth.

25.2.37. She complained of headache and hunger. Blood pressure 95/70. Heart : regular.

1.3.37. Discharged well.

Blood and Urine Examinations :—

Blood	11.15 a.m.,	sugar	0·230	per cent.
„	11.45 a.m.,	„	0·208	„
„	12.15 p.m.,	„	0·158	„ Urea, 0·027 per cent.
„	1.20 p.m.	„	0·130	„
„	2.30 p.m.,	„	0·98	„
„	3.30 p.m.,	„	0·84	„ Chloride, 0·496 per cent. as NaCl.
Urine,	10.30 a.m.,	„	2·0	„
„	11.45 a.m.,	„	1.2	„ Albumin +++.
„	12.15 p.m.,	„	trace.	„ ++.
„	1.40 p.m.,	„	„	„ +.
„	2.30 p.m.	„	nil	„ trace.

Case 7.—*R., female, age* 11 *years. A case of " dystonia musculorum deformans " or torsion spasm precipitated by scorpion sting.*

Admitted 4.10.33 with the following history : that she was quite well until 4.9.33. She was stung by a scorpion that day, after which she vomited and at 10 p.m. began to get convulsions but did not lose consciousness. She was taken to a district hospital the same evening and remained there 19 days, then removed from hospital as her condition did not improve, and while at home her body became twisted. She was then brought to the hospital at Port of Spain. Notes at the hospital were as follows :—

Admitted 4.10.33. Temperature 105° F., and having convulsions. An injection of quinine was given and the temperature dropped. There was paralysis of the left side of the body. On admission on 4.10.33 examination revealed : heart and lungs : nil ; liver and spleen : not enlarged, somewhat anaemic. She was quite intelligent and bright mentally. There was extreme retraction of the head and hyperextension of the spine, especially in the lumbar region. Opisthotonus and pleurosthotonus of the right side of the body. The right lower limb was extended and kept rigid. The left lower limb was flexed at the knee with foot dorsi flexed. Waist acutely flexed. Right upper limb adducted flexed at elbow and wrist. Every few seconds there were spasmodic contractions of the whole body. The muscles were never completely relaxed and readily contracted on the slightest stimulation. Babinski was positive.

Blood Examination : Polymorphs 54 per cent. ; Large mononuclears 4·2 per cent. ; eosin 20 per cent. ; Large lymphocytes 2.3 per cent. ; Small lymphocytes 19·2 per cent.

Urine : no casts, no blood, pus cells and Gram negative bacilli.

Wasserman : positive ++.

Blood calcium : 6.10.33, 10·7 mg. per 100 c.c. ; 9.10.33, 9·8 mg. per 100 c.c.

Stools : contained ova of *Ankylostoma, Trichocephalus dispar* and *Ascaris*. The patient was treated for the worms with chenopodium.

One of the characteristics of the condition was that she would cry out loudly, especially at nights. She was quite conscious of making the noise but was unable to control herself. This necessitated a hypnotic to procure for her and the other patients some sleep. She had to be fed on liquids and very slowly for swallowing was difficult. Her sphincters and sensation were normal. Fright or excitement aggravated and intensified the muscular contractions.

Case 8.—*J. J., age* 8 *years showing signs and symptoms of acute pancreatitis.*

History : Stung on the left thumb 2.10.37.

On admission to hospital at 9 a.m. she was sweating profusely and vomiting frequently. She complained of much abdominal pain. T. 98°, P. 100, R. 34.

On examination : Heart and lungs : nil abnormal to note. Abdomen : very tender.

3.10.37. Abdomen distended, tympanitic and very tender especially in epigastric region. 2 p.m., T.100·4°, P. 120, R. 30.

4.10.37. 6 a.m , T. 100·6°, P. 148, R. 36. Diastatic index urine = 67 ; Blood: R.B.C. =4,370,000; W.B.C. = 14,027; Hb. = 78·6 per cent.; Differential : neutrophils 80 per cent., small lymphocytes 8 per cent., large lymphocytes 9 per cent., large hyaline 3 per cent. 10 p.m., T. 100·6°, P. 140, R. 32.

5.10.37. 6 a.m., T. 98°, P. 120, R. 32. Abdomen less distended. Patient states that the abdomen is less painful. 2 p.m., T. 100·2°, P. 136, R. 36.

6.10.37. 6 a.m., T. 97·4°, P. 108, R. 24. Patient made an uninterrupted recovery.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

THE CEREBROSPINAL FLUID OF PATIENTS SUFFERING FROM THE CHINESE STRAIN OF RELAPSING FEVER.

BY

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There are a few reports concerning observations on the cerebrospinal fluid of patients suffering from either the European or the African variety of relapsing fever. The present communication deals with the cerebrospinal fluid findings of the victims of that disease in China, besides recording an unusual phenomenon, namely, the exceptionally high incidence of transient but clear-cut positive Wassermann reaction of the cerebrospinal fluid of Chinese relapsing fever patients.

MATERIAL.

Altogether the cerebrospinal fluids of 35 patients with relapsing fever were studied. Of the 35 patients, 9 had a positive blood Wassermann reaction and one of the 9 also had a positive cerebrospinal fluid Wassermann reaction. As these 9 patients were considered to have syphilis, they are not included for analysis here (except one whose cerebrospinal fluid had been inoculated into a squirrel). Hence the present report deals only with the findings of 26 patients. Of these 26 patients, 9 had 1, 10 had 2, 6 had 3, and 1 had 4 examinations of the cerebrospinal fluid, the first specimen of which was obtained when the blood contained numerous spirochaetes, except in 5 cases in which lumbar puncture was done during crisis or remission.

METHOD.

The technique of lumbar puncture is so well known that it requires no description here. The pressure of the cerebrospinal fluid was noted, and care was taken to ascertain whether or not there was any obstruction to the flow of the fluid. The fluid was collected aseptically in a sterile test tube for study. The colour and transparency of the fluid were noted and the cellular elements were counted. To determine whether or not there was any increase in the proteins, the fluid was subjected to Pandy and Nonne tests. In a few instances, the total proteins in the fluid were determined quantitatively, but the results

Case No.	Age : Sex.	Date of Lumbar Puncture.	Lumbar puncture in Relation to Treatment.	Duration of Illness in Days.	Patient's Rectal Temp. ° C.	*S. recurrentis* in Patient's Blood.	Cerebrospinal Fluid.							
							Pressure in mm. H_2O.	Appearance.	R.B.C. per c.mm.	W.B.C. per c.mm.	Pandy.	Nonne.	Kolmer's Wassermann.	Colloidal Gold Curve.†
1	22 M.	May 16	Before N.A.B.	4	39·2	+	70	Normal	0	4	0	0	444--	Normal
		„ 18	After N.A.B.		37·2	0	150	„	0	2	0	0	144—	„
		„ 25			37·6	0		Slightly hazy	740 (Trauma)	2	0	0	-----	„
		„ 29			37·7	0	125	Normal	6	0	0	0	-----	„
2	14 M.	„ 12	Before N.A.B.	14	40·6	+	110	„	0	4	0	0	-21--	„
		„ 18	After N.A.B.		37·3	0	160	„	0	12	0	0	-----	„
		„ 30			37·4	0	190	„	0	8	0	0	-----	„
3	16 M.	„ 25	Before N.A.B.	4	39·8	+	170	„	0	0	0	0	445--	„
		„ 29	After N.A.B.		37·0	0		Slightly pinkish	6,400 (Trauma)	18	T	T	-----	„
		June 4			37·6	0	150	Xantho-chromic	48	12	T	T	-----	„
4*	15 M.	Apr. 22	No treatment	15			130	Normal	0	4	0	0	-----	„
		May 29	„		37·2	0	140	„	0	4	0	0	-----	„
		June 3	„		37·7	0	190	„	0	0	0	0	-----	„
5	17 M.	May 19	Before N.A.B.	3	40·1	+	70	„	0	6	0	0	-44--	„
		„ 26	After N.A.B.		37·4	0	150	„	5	0	0	0	-----	„
		„ 29			37·4	0	70	„	160	10	0	0	-----	„

6	33 M.	„ 12	16 days after N.A.B.	22	38·4	0	116	Normal	32	72 Lym.	+	+	-----	Normal
		„ 25			38·4	0	150	„	2	5	T	T	-----	„
		June 1			38·0	0		„	0	0	0	0	-----	„
7*	25 M.	Apr. 10	Before N.A.B.	4	40·6	+	100	„	0	2	0	0	-44± -	„
		April 24	Before		37·4	0	120	Slightly pinkish	1,350 (Trauma)	12	T	0	-----	„
		May 25	After N.A.B.		37·2	0	120	Normal	0	0	0	0	-----	„
8	19 M.	„ 12	Before N.A.B.	12	39·8	+	120	„	320	32	T	0	2444-	„
		„ 26	After N.A.B.		37·4	0	190	„	5	0	0	0	-----	„
9	33 M.	Apr. 14	Before N.A.B.	8	40·0	+	130	„	0	4	T	0	-32--	„
		„ 18	After N.A.B.		37·4	0		„	12	82	0	0	-----	„
10	46 M.	May 26		5 days before relapsing fever	37·0	0	100	„	20	0	0	0	-----	
		June 2	Before N.A.B.	2	41·0	+	30	„	0	12	0	0	-----	Normal
11	30 M.	Apr. 30	Before N.A.B.	8	38·0	+		Icteric	0	4	0	0	-412-	„
		May 18	After N.A.B.		37·2	0	170	Normal	0	35	T	0	-----	„
12	34 M.	„ 13	Before N.A.B.	5	40·3	+	150	„	6	2	0	0	--31±	„
		„ 20	After N.A.B.		37·2	0	190	„	25	2	0	0	-----	„

(*Continued on next page*)

TABLE I—(*continued*).

Case No.	Age: Sex.	Date of Lumbar Puncture.	Lumbar puncture in Relation to Treatment.	Duration of Illness in Days.	Patient's Rectal Temp °C.	*S. recurrentis* in Patient's Blood.	Cerebrospinal Fluid.							
							Pressure in mm. H_2O.	Appearance.	R.B.C. per c.mm.	W.B.C. per c.mm.	Pandy.	Nonne.	Kolmer's Wassermann.	Colloidal Gold Curve.†
13	14 M.	July 22	Before N.A.B.	4	39·8	+	150	Normal	13	32	0	0	-----	Normal
		„ 26	After N.A.B.		37·4	0	122	„	0	4	0	0	-----	„
14	16 M.	„ 1	Before N.A.B.	24	40·6	+	140	„	0	6	0	0	-----	„
		„ 3	After N.A.B.		36·4	0	114	„	12	2	0	0	-----	„
15	14 M.	„ 8	Before N.A.B.	3	39·4	+	150	„	0	5	0	0	-----	„
		„ 15	After N.A.B.		37·2	0	125	„	0	20	0	0	-----	„
16	15 M.	May 26	Before N.A.B.	4	39·0	+	110	„	0	5	0	0	-----	„
		„ 30	After N.A.B.		37·4	0	130	„	0	8	0	0	-----	„
17	15 M.	„ 21	Before N.A.B.	24	40·2	+	105	„	0	2	0	0	±3---	„
		„ 26	5 days after N.A.B.		36·8	0								

18	34 M.	Mar. 3	4 days after N.A.B.	30±	37·2	0	120	Normal	0	2	+	0	-----	000122100											
19	38 M.	,, 11	No treatment	11	37·2	0 Natural crisis	?	,,	0	11	0	0	-----	Normal											
20*	30 M.	Apr. 13	Before N.A.B.	11	40·3	+	90	,,	0	0	0	0	-----												
21	19 M.	May 29	Before N.A.B.	5	39·6	+	211	,,	0	2	0	0	2----	,,											
22	13 M.	,, 28	Before N.A.B.	49	39·3	+	142	,,	0	4	0	0	-----	,,											
23	20 M.	Apr. 28	Before N.A.B.	2	39·0	+	212	,,	196	4	0	0	-----	,,											
24*	29 M.	May 8	Before N.A.B.	8	38·2	+	160	,,	0	4	0	0	-----	,,											
25*	28 M.	Apr. 6	Before N.A.B.	20	38·8	+	60	,,	36	20	0	0	-----	,,											
26	47 M.	June 6	5 days after N.A.B.	13	37·2	0	60	,,	0	6	0	0	-----	,,											

Note.—Blood Wassermann and Kline tests were negative in all cases.

* Cerebrospinal fluid was examined under dark-field microscope and also inoculated into squirrels. See Table II.

N.A.B. = Neo-arsenobenzol.

† The writer is indebted to the Department of Bacteriology and Immunology for the results of the Wassermann and colloidal gold tests included in this table,

are not included here as they were within normal limits. Without exception specimens of the fluid were subjected to Wassermann and colloidal gold tests to determine their significance in relapsing fever. In order to determine the infectivity of the fluid, 7 specimens from 6 patients (Table II) were injected into splenectomized normal squirrels. Six of these 7 specimens were also centrifuged and their sediments were examined under the dark-field microscope for spirochaetes. When a lumbar puncture was done, a specimen of blood from the patient was obtained for Wassermann and Kline tests.

Results.

I.—Physical properties of cerebrospinal fluid.

(*a*) *The pressure of the cerebrospinal fluid* was determined 44 times in 26 patients. It varied from 150-190 mm. of water in 16 instances and exceeded 210 mm. in two instances. In the remaining 26 instances it fluctuated between 30 and 140 mm.

(*b*) *The appearance of the cerebrospinal fluid* was always transparent, clear and colourless except in three instances in which it was hazy or pinkish due to haemorrhage from trauma during the puncture, in one instance in which it was icteric because the patient had jaundice, and in another instance in which it was xanthochromic because of haemorrhage from a previous puncture.

(*c*) *The leucocytes* in the cerebrospinal fluid were counted in 50 specimens from 26 patients. Their number varied from 8 to 20 per c.mm. in 16 specimens, from 32 to 82 in 4 specimens, and from zero to 6 in the remaining 34 specimens. The predominating white cell in all instances in which a differential count was done was the lymphocyte.

(*d*) *The erythrocytes* in the cerebrospinal fluid were counted in 50 specimens. The count, which was zero in 31 specimens, varied from 2 to 25 per c.mm. in 10, from 30 to 50 in 3, from 100 to 400 in 3, and from 600 to 7,000 in 3 specimens. In the instances in which the cerebrospinal fluid contained considerable numbers of erythrocytes trauma was found to be the cause.

II.—Chemical tests.

(*a*) *Pandy's* and *Nonne's tests* were performed on 50 specimens from 26 patients. Pandy's test was faintly positive in 8 specimens of which 4 showed increased R.B.C. due to trauma from puncture. Nonne's test was positive in 4 specimens, of which 2 showed increased R.B.C. due to trauma from puncture.

III.—Serological reactions.

(*a*) *The Wassermann test* was done on 50 specimens from 26 patients. Of 16 patients whose cerebrospinal fluid was subjected to the Wassermann test from 2 to 4 times, 9 showed a transient but clear-cut positive reaction which

TABLE II.

Case No.	Date of Examination.	Duration of Patient's Illness.	Patient's Temperature in ° C.	*S. recurrentis* in Blood (Dark-field).	*S. recurrentis* in C.S.F. (Dark-field).	Squirrel Inoculation.		
						Dose and Route.	Squirrel No.	*S. recurrentis* in Blood.
25*	April 6	21 days; 2nd day of 2nd attack	38·8	+	0	2 c.c. I.P. 1 c.c. I.V.	171	+ (10th day)
7*	,, 10	21 days; 2nd day of 2nd attack	40·6	+	Not examined	Ditto	177	+ (10th day)
20	,, 13	11 days; 1st attack	38·5	+	0	Ditto	171	+ (9th day)
27**	,, 14	5 days; 1st attack	39·8	+	0	Ditto	181	+ (8th day)
24*	May 8	8 days; 1st attack	38·1	+	0	Ditto	183	0
4*	Apr. 22	23 days	38·2	0	0	Ditto	188	+ (12th day)
	June 3	Recovered		0	0	Ditto	214	0

* See Table I for details.

** This patient had a positive blood Wassermann reaction (due to syphilis or relapsing fever ?). Hence his spinal fluid findings were not included in Table I.

I.P. = Intraperitoneal. I.V. = Intravenous. C.S.F. = Cerebrospinal fluid.

suddenly became completely negative 4 to 18 days subsequently. Of the 10 patients whose cerebrospinal fluid was subjected to the test only once, 2 gave a positive reaction.

With every Wassermann test of the cerebrospinal fluid, Wassermann and Kline tests were done on the blood from the patient at the same time. The reactions of the blood were always negative. In several instances a Kahn test of the blood was also performed which showed a transient positive reaction in one case (Case 17).

(*b*) *The colloidal gold curve test* was performed on 50 specimens from 26 patients. In every instance the test was uniformly negative, except in Case 19 in which the test might be considered to have given a slightly abnormal curve; see Table I.

IV.—SPIROCHAETES IN AND INFECTIVITY OF CEREBROSPINAL FLUID.

Altogether 7 specimens from 6 patients were studied. Six of these which were examined under the dark field microscope showed no spirochaetes. When inoculated into normal but splenectomized squirrels 5 of the 7 specimens caused relapsing fever (see Table II). One of the 2 specimens which failed to produce infection in a squirrel was obtained when the patient had no fever (spontaneous recovery).

Discussion.

As seen in Table I, in many instances the leucocytes in the cerebrospinal fluid were increased and occasionally the pressure of the fluid was augmented. These changes, although not very marked, seem to indicate that in relapsing fever the central nervous system is more or less affected, at least in some cases. Petzetakis (1916) and Cawadias (1921) observed increased cerebrospinal fluid pressure in cases of relapsing fever showing a syndrome not unlike that of meningo-encephalitis, but the leucocytes in the cerebrospinal fluid in their series were not increased. Babes (1916) reported haemorrhagic meningitis in relapsing fever. The fact that the spinal fluid is infectious, as shown by squirrel inoculation in our series (see Table II), means that *Spirochaeta recurrentis* actually invades the central nervous system. Indeed, as early as 1907, Soulie reported the presence of numerous spirochaetes (species not determined; *S. duttoni* or *S. obermeieri*?) in the cerebrospinal fluid of a patient showing meningeal symptoms during relapsing fever. The cells in the fluid were chiefly lymphocytes but occasionally polynuclear leucocytes. In 1914, Brault and Montpellier recovered *S. duttoni* from 2 of the 20 specimens of the cerebrospinal fluid from 20 patients subjected to a lumbar puncture. Similar observations were made by Wiener (1917) on cases of relapsing fever in Albany, by Leboeuf and Gambier (1918) on cases of relapsing fever in the Congo, and by Nitzescu (1921) on patients suffering from *S. obermeieri*. In 1919 Plaut and Steiner

found that the cerebrospinal fluid of 5 of 10 patients suffering from general paralysis of the insane, under treatment with the African variety of relapsing fever (*S. duttoni*), was capable of producing infection (relapsing fever) in susceptible animals and remained infectious, in one instance as long as 69 days after the therapeutic infection, when the patient's blood was already non-infective. Upon careful examination of one human brain by JAHNEL (1926) and another by JAHNEL and LUCKSCH (1927), *S. duttoni* and *S. obermeieri* respectively were found in the parenchyma of the two brains examined. Besides clinical observations, many observers including BUSCHKE and KROÓ (1923), TOMIOKA (1924), JOHANNESSHON (1926), KRITSCHEWSKI (1927), PRIGGE and ROTHERMUNDT (1928), and SEYFARTH, SARAFOFF and KUSSITASSEFF (1925) brought forth experimental evidence to show the involvement of, and the persistence of infection in, the brains of infected susceptible animals like rats and mice, and concluded that the organisms were by no means exclusively blood parasites. Obviously *S. duttoni* is more neurotropic than *S. obermeieri*.

The most striking finding in our series, however, is the unusually high incidence of transient but definitely positive Wassermann reactions as shown in Table I. This has never been observed before. Without further investigation we hesitate to offer any explanation at present, although it appears certain that it is related to the relapsing fever infection, particularly in view of the fact that the blood of patients with Chinese relapsing fever not rarely gave a false positive Wassermann, Kahn, or Kline reaction as shown by T'UNG and CHUNG (1937). None of the 26 patients in the present series had any history or physical evidence of syphilis. The Wassermann and Kline reactions of their blood were uniformly negative.

SUMMARY AND CONCLUSIONS.

1. The cerebrospinal fluids of 26 patients with relapsing fever have been studied.

2. The physical properties of these fluids were normal in most instances. In some instances there was a definite increase in the leucocyte counts, chiefly in lymphocytes, and in a few instances the pressure of the fluid was somewhat augmented.

3. Pandy's and Nonne's tests were weakly positive in several instances, but the colloidal gold test was negative, except in one instance in which it was slightly abnormal.

4. Of 16 patients whose cerebrospinal fluid was subjected to the Wassermann test from 2 to 4 times, 9 showed a transient but clear-cut positive reaction which suddenly became completely negative 1 to 3 weeks later. Of the 10 patients whose cerebrospinal fluid was tested only once, 2 gave a positive Wassermann reaction.

5. The transient positive Wassermann reactions of the cerebrospinal fluid are thought to be related to the relapsing fever, from which the patients were suffering.

6. Seven specimens of cerebrospinal fluid from 6 relapsing fever patients were inoculated into 7 squirrels, of which 5 developed relapsing fever.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No 6. April, 1938.

THE DEATH RATE AND EPIDEMIOLOGY OF SMALLPOX IN HONGKONG.

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INTRODUCTION.

This paper will be concerned with smallpox as it occurred in Hongkong, from 1897 to 1936 inclusive, among the resident urban Chinese of the Colony. The data have been collected by a personal inspection of all death registrations occurring during the period in question among the city dwellers of Victoria and Kowloon. For information about the structure of the urban Chinese population of the Colony reference must be made to my previous papers, UTTLEY (1938*a* and 1938*b*) dealing with death rates from tuberculosis, and from all causes, where the material will be found for comparing Hongkong statistics with those from other places. It is not necessary to repeat it here.

My data will be considered under the following headings :—

(*a*) Standardized and crude death rates for Hongkong.
(*b*) Age incidence of deaths.
(*c*) Seasonal mortality.
(*d*) Vaccination and its effect on the age incidence of deaths.
(*e*) Coefficients of correlation between certain climatic factors and the monthly mortality.
(*f*) A comparison of (*e*) with what has been found to apply to India.
(*g*) Conclusions and summary.

(*a*) STANDARDIZED AND CRUDE DEATH RATES FOR HONGKONG.

Smallpox has been present in Hongkong during the British occupation for as long as there are records. Early records are, however, incomplete and I propose to commence with the year 1897, because it was not until that year

that a census was taken which was sufficiently accurate to enable the epidemiologist to calculate standardized death rates. I have calculated the standardized death rate for each census year of the period under discussion, basing my calculations on the standard British population of 1901, using the direct method. For intercensal years I have given the crude rate only, because the population of the urban areas of the Colony is too shifting in its nature to permit of a standardized rate being calculated for intercensal years. Hitherto no attempt to calculate the mortality of the disease has been made other than to state the crude rate for the Colony as a whole, and this includes Europeans, Eurasians, many thousands of inhabitants of junks and sampans and country folk, besides the urban Chinese discussed in this paper.*

Table I shows the death rates from smallpox. It will be seen that in the census years, where standardized rates can be calculated, four out of the six years show a female rate exceeding the male rate in the proportion of three females to two males, and that in the other two years, the male rate has exceeded the female one by three to two. The table also shows that a year with a high mortality is usually succeeded by several years with much lower rates. Smallpox is a compulsorily notifiable disease, but notification is largely ignored. In an average year, such as for instance 1933, 69·3 per cent. of all smallpox cases were notified by the medical officers in charge of the public mortuaries, *i.e.* after death had occurred, and the Director of Medical Services has calculated that over 67 per cent. of all cases of smallpox avoid the notice of the Sanitary Authority and are never notified (WELLINGTON, 1933). A major reason for this is that Chinese do not consider smallpox to be a serious disease, and are allowed to treat the condition by native herbalist methods. The degree of success of these methods is shown by the comparative statistics of a hospital run on native lines, where in 21 years 1,249 cases were treated for smallpox with a mortality of 46·8 per cent., whereas the Government Infectious Fevers Hospital during the same years dealt with 288 cases with a mortality of 14·2 per cent. (WELLINGTON, 1932).†

(*b*) AGE INCIDENCE OF DEATHS.

Table II shows the deaths at ages per 1,000 living at ages; British figures for 1901 have been added for comparison. For the first five census years the mortality fell upon the younger age groups much more heavily than it did in England and Wales, owing to the relatively large numbers of vaccinated children in the latter country.

* The disease as met with in Hongkong has always been of the variola major form. It is true that chickenpox is sometimes mistaken for smallpox, but the mortality from the former is negligible, and any error so introduced, may for the purpose of this paper be overlooked.

† N.B.—The standard error of the difference between these two percentages is 2·495.

SMALLPOX IN HONGKONG, 1897 TO 1936. STANDARDIZED AND CRUDE DEATH RATES.

TABLE I.

Year.	Standardized death rates.			Crude death rates.	Urban population.
	Males.	Females.	Persons.	Persons.	
1897				1·05	
1898	1·42	2·33	1·82	0·44	163,075
1899				0·09	
1900				0·08	
1901					
1902	0·37	0·51	0·44	0·23	
1903				0·16	220,319
1904				0·11	
1905				0·12	
1906				0·06	
1907	0·79	0·54	0·66		
1908				0·37	
1909				0·79	239,852
1910				1·13	
1911				0·05	
1912	1·06	1·55	1·31	0·04	
1913				0·66	
1914				1·05	286,118
1915				0·23	
1916				0·26	
1917				0·06	
1918				1·42	
1919				1·34	
1920				0·05	
1921				0·03	
1922	0·39	0·51	0·45	0·04	
1923				0·34	
1924				0·36	434,724
1925				2·27	
1926				1·51	
1927				0·08	
1928				0·05	
1929				0·20	
1930				0·51	
1931				1·58	
1932	0·021	0·014	0·018	0·37	
1933				0·01	
1934				0·25	640,746
1935				0·68	
1936				0·16	
				0·06	
				0·01	
Mean crude death rate				0·46	

Except in 1931, when the figures were too small for accurate comparison, at ages 0 to 4 years, the female rate is always higher than the male one, as in Britain in 1901. At 5 to 9 years of age, the male and female mortality figures, though they vary a good deal from one census year to the next, bear a closer relationship to each other than do the British ones for 1901. In the group aged 15 to 19 years the

TABLE II.

DEATHS FROM SMALLPOX AT AGES PER 1,000 LIVING AT SUCCESSIVE CENSUS YEARS.

(Figures for England and Wales for 1901 given for comparison.)

1897.		1901.		1906.		1911.		1921.		1931.		England and Wales, 1901.	
M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
6·25	13·50	2·05	3·47	5·70	5·72	7·37	11·39	2·52	3·47	0·15	0·04	0·025	0·034
3·13	3·25	0·40	0·53	0·95	0·72	0·96	1·29	0·52	0·60	—	—	0·017	0·009
1·51	1·62	0·27	0·16	0·13	0·61	0·51	0·41	0·10	0·11	—	0·04	0·008	0·014
0·51	0·52	0·19	—	0·05	0·52	0·09	0·55	0·08	0·19	—	—	0·012	0·008
0·62	0·47	0·11	0·16	0·35	0·26	0·10	0·64	0·16	0·18	0·02	—	0·015	0·013
0·53	—	0·09	0·16	0·06	—	0·11	0·05	0·11	0·03	0·01	0·02	0·035	0·030
0·29	0·29	0·11	—	0·10	0·10	0·14	0·14	0·04	0·04	—	—	0·045	0·029
0·08	0·61	—	—	—	0·17	0·11	0·13	0·04	—	—	—	0·029	0·011
—	—	—	—	—	—	0·11	0·16	—	—	—	—	0·009	0·006
												0·006	0·001

male rate has been usually much less than the female one, this being the reverse of the British figures for 1901. In each successive male age group from 15 to 34 there is a moderately constant death rate in any one census year (though varying considerably from one census year to the next, depending on the severity of smallpox), which is in marked contrast with the tripling of the rate in England and Wales for the same age groups in 1901. The Hongkong female rate, on the other hand, usually fell steadily from 15 to 34 years of age, again in contrast to the 3½ times increase in the corresponding British rate for 1901. Figures for 35 years of age and over are too small for any deduction to be made from them, though it is of interest to note that except for one year the male rate at ages 35 to 44 has always been the same as the female one.

In considering the age incidence of deaths it is necessary to bear in mind the different composition of the population in Hongkong from that of England and Wales. Details of this are given in my paper, UTTLEY (1938*b*) on death rates in Hongkong, but briefly it may be said that there was a smaller proportion

of children in the Hongkong population than in the British one until 1921. From then onwards the Hongkong percentage was a fraction higher than the British one. In the working years of life, from 15 to 44 years of age, the percentage has always been much higher in Hongkong than in Britain. This is partly owing to the immigration of able-bodied adults seeking work, partly to the very much shorter expectation of life among Chinese, and partly to the fact that when the Chinese have earned a little money, a certain unknown proportion return to their up-country villages to settle down.

Table III shows the percentage of all deaths from smallpox occurring in each age group for the different intercensal periods, and for the total period 1897 to 1936.

TABLE III.

PERCENTAGE OF ALL DEATHS FROM SMALLPOX OCCURRING IN EACH AGE GROUP, 1897-1936.

Age in years.	1897-1900.			1901-1910.			1911-1920.			1921-1930.			1931-1936.			1897-1936.
	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	M.	F.	P.	Persons.
0–	5·3	9·8	7·8	5·1	6·7	5·9	10·2	12·0	11·2	13·8	14·9	14·4	15·1	19·5	17·4	12·82
1–	8·3	12·9	10·8	11·7	16·2	13·9	15·7	21·0	18·6	18·7	21·4	20·0	13·5	20·5	17·1	18·41
2–	6·8	17·8	12·9	16·6	24·4	20·5	23·1	25·4	24·4	24·0	23·2	23·6	17·4	13·7	15·5	22·18
3–	5·3	12·3	9·2	11·7	12·6	12·1	11·9	10·2	11·0	12·5	16·5	14·5	10·9	12·2	11·6	12·91
4–	4·5	5·5	5·1	7·8	7·7	7·7	5·3	6·1	5·7	5·8	5·1	5·5	4·6	6·3	5·5	5·76
0–4	30·3	58·3	45·8	53·0	67·4	60·3	66·2	74·8	70·9	74·8	81·2	78·0	61·7	72·2	67·1	72·09
5–	4·5	4·9	4·7	4·7	3·8	4·3	1·9	3·1	2·6	2·8	2·5	2·7	3·4	2·7	3·0	2·92
6–	4·5	5·5	5·1	4·4	4·1	4·3	2·1	1·4	1·7	1·7	1·1	1·4	1·8	2·2	2·0	1·99
7–	5·3	3·7	4·4	0·8	1·8	1·3	1·1	1·1	1·1	0·8	1·7	1·2	0·5	1·7	1·1	1·31
8–	0·8	3·1	2·0	2·9	2·3	2·6	0·3	1·0	0·6	1·3	1·0	1·2	0·8	0·2	0·5	1·16
9–	0·8	1·2	1·0	0·5	—	0·3	0·3	0·8	0·5	0·4	0·3	0·3	—	0·7	0·4	0·40
5–9	15·9	18·4	17·3	13·2	12·1	12·6	5·7	7·3	6·6	6·9	6·6	6·8	6·5	7·6	7·0	7·77
10–	6·8	7·4	7·1	4·2	5·4	4·8	3·4	5·0	4·3	1·9	2·8	2·3	1·6	2·5	2·0	3·20
15–	7·6	8·6	8·1	5·1	4·1	4·6	4·3	2·6	3·4	3·6	1·7	2·6	6·8	2·7	4·6	3·44
20–	12·9	2·4	7·1	5·9	4·1	5·0	5·6	3·8	4·6	4·0	2·5	3·3	9·1	4·9	6·9	4·30
25–	19·7	1·8	9·9	11·1	4·9	8·0	9·0	4·6	6·8	5·8	3·5	4·6	8·6	6·1	7·3	5·97
35–	5·3	1·2	3·1	6·5	1·6	4·0	3·7	1·6	2·6	1·9	1·4	1·7	3·6	3·2	3·4	2·35
45–	1·6	1·8	1·6	0·8	0·3	0·5	1·7	0·2	0·9	0·8	0·3	0·6	1·8	0·7	1·3	0·71
55–	—	—	—	—	—	—	0·2	0·1	0·2	0·1	0·2	0·1	0·3	0·2	0·3	0·13
65–	—	—	—	0·3	0·3	0·3	0·1	—	0·1	—	—	—	—	—	—	0·04
75+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	132	163	295	385	390	775	791	932	1,723	1,976	1,924	3,900	384	410	794	7,487

Males.—At ages 0 to 4 there was a steady rise from 1897 to 1930 in the percentage of those dying in this age group, the figure having more than doubled in that time. In the last 6 years however, the figures dropped from 74·8 per cent. to 61·7 per cent. As will be seen later, this fall is due to the intense vaccination campaigns instituted since 1929. In the age group 5 to 9 years, the initial percentage of 15·9 fell to 5·7 in 1911 to 1920, to rise a little since. In the next group there has been a continuous fall. In the groups aged 20 to 34 years, there was a fall until 1930, and then a rise. From 35 to 54 years of age there were irregular fluctuations in the percentage, while at ages above this, there were too few deaths to be able to infer any changes.

Females.—At 0 to 4 years of age the changes were similar to those occurring among males of the same age, but the percentage was consistently higher than in the corresponding male group. At 5 to 19 years, there was a steady fall until 1921-1930, after which there was a rise. In the next age group there was an irregular fluctuation in the figures. At 25 to 44 there was on the whole an irregular rise throughout the period. At 45 to 54 years, the initial figure of 1·8 per cent. fell to a steady low figure of about 0·2 per cent. until the last 6 years, when it rose to 0·7 per cent. There were very few deaths at ages of 55 years and over.

Comparison of male with female mortality.—Until 14 years of age the female rate is higher than the male rate except at 5 to 9 years of age in 1901-1910 and 1911-1920. At higher ages, *i.e.*, during the working years, the male rate almost always exceeds that of females.

This table emphasizes the fact of the heavy mortality from smallpox in the first 5 years of life, amounting to over three-quarters of all smallpox deaths in the 10-year period 1921-1930. In this respect the mortality resembles that which occurred in England in the eighteenth century, before the introduction of vaccination, when the death rate under 5 years was 80 per cent. of all smallpox deaths (McVail, 1923).

(*c*) Seasonal Mortality.

Table IV shows the seasonal mortality from smallpox. As elsewhere in the world, it is essentially a winter and spring disease, but there are sporadic cases throughout the year. Deaths usually begin to be reported in numbers in November, but epidemics are not expected until January, after which they last until the end of April.

It is worth while to point out in this connection a custom of the Chinese of South China, which undoubtedly has an influence on the spread of the disease, and it is that as soon as winter is over, a large proportion of the population take all their winter clothes to the pawnbroker for safe keeping until the beginning of the cold weather. As these clothes are not disinfected, aired or in any way treated for any fomites, but are packed away on shelves, close to others, smallpox infection is probably still present in the garments when they are taken

out of pawn, thus tending to spread the disease when they are worn again. Winter clothes are not changed very often, and as members of the family huddle together at night for warmth, the spread of the disease is further facilitated.

TABLE IV.

AVERAGE MONTHLY AND QUARTERLY MORTALITY FROM SMALLPOX, HONGKONG; 1897-1936.

Month.	Percentage of all smallpox deaths.	Month.	Percentage of all smallpox deaths.
January	17·3	April	14·2
February	17·0	May	7·7
March	15·7	June	4·1
First quarter	50·0	Second quarter	26·0
July	1·7	October	2·3
August	1·1	November	5·9
September	0·9	December	12·1
Third quarter	3·7	Fourth quarter	20·3

Total smallpox deaths 7,487

(*d*) VACCINATION AND ITS EFFECT ON THE AGE INCIDENCE OF DEATHS.

Until 1928 there was very little vaccination carried out in the Colony. During the years 1910-1920 there was an average of 15,000 every year. From then until the end of 1927 the average rose somewhat irregularly and slowly. In 1928 the matter was taken up with certain first aid societies and other bodies in order to have annual campaigns to interest the population in the question, with the result that a steady average of well over 300,000 have been vaccinated annually since, equal to $2\frac{1}{2}$ times the population of the Colony. It must be remembered, however, that a large minority of the urban population is constantly travelling to and from Hongkong and China, so that there is always a reservoir of unvaccinated people in the Colony. Chinese do not object to vaccination, on the contrary, they welcome it when they see its advantages, but they object to infants being vaccinated until they have passed their second Chinese New Year. "A child born just after the Chinese New Year is thus 2 years of age before it is vaccinated. In spite of the law requiring children to be vaccinated within 6 weeks of birth, many remain undone until the so-called propitious period, and thus there is always in the Colony sufficient suitable soil for the growth and development of the smallpox virus" WELLINGTON (1933). As soon as the Chinese New Year is over the dispensaries and hospitals of the city are crowded with mothers bringing their infants to be vaccinated. I consider that this local prejudice is the reason for the small fall in the mortality below

2 years of age seen in Table III. The extensive vaccination campaigns of the last few years have altered the mortality at ages in an interesting way. If reference is made again to Table III, it will be seen that in the years after 1930 a fall of 11 per cent. has occurred in the " persons " group aged 0 to 4 years, that there is little change at ages 5 to 14, and that above 14 the percentage has risen. This rise is largely a relative one, because it is children who have been vaccinated in proportionately larger numbers than adults, thus leaving a relatively larger proportion of adults to be attacked by the disease.

If the deaths occurring in each intercensal period are investigated, and the percentage at ages 0 to 4 examined, it will be seen that the steady increase from 1897 to 1930 became arrested, and fell during 1931-1936. The differences between the figures of one intercensal period and the next are all statistically significant, and I consider that it is right to infer that the decrease after 1931 must be due to vaccination, because I have been unable to discover any other factor or group of factors that might have caused this change.

It is as yet unwise, however, to draw any conclusion as to whether the low crude death rates met with since 1930 are due to the intense vaccination campaigns or not (though they probably are due to them), because population estimates, and therefore crude death rates calculated from them, may be somewhat inaccurate owing to such economic factors as the recent slump affecting the numbers of certain elements of the population to a greater or less extent than has been allowed for.

Fatality rates among the vaccinated and unvaccinated.—Unfortunately the returns are not sufficiently complete to allow of a determination of the different fatalities among the vaccinated and unvaccinated.

(*e*) Coefficients of correlation between certain climatic factors and the monthly mortality.

I have calculated the coefficients of correlation between the four main climatic factors, namely rainfall, temperature, relative humidity and pressure, and smallpox mortality for the 40 years under discussion. The total frequency for each factor being 480, the standard error in each case is 0·04569, assuming S.E. is $\frac{1}{\sqrt{n-1}}$ These values are shown in Table V.

In the first column of figures, the climatic factor is correlated with the smallpox mortality for the same month. In order to test whether the maximum mortality from smallpox coincided with the peaks of the various climatic factors or whether there was a lag of 1 month or more between the two, I have used Russell and Sundararajan's (1929) method of calculating these delays for the disease in India. The second column of figures shows the mathematical values for a delay of 1 month between the climatic factor and smallpox deaths, *i.e.*, January pressure figures are correlated with February mortality, February

TABLE V.

COEFFICIENTS OF CORRELATION BETWEEN CERTAIN CLIMATIC FACTORS AND SMALLPOX, HONGKONG, 1897-1936.

Climatic factor.	Lag_0	Lag_1	Lag_2	Lag_3
Atmospheric pressure	0·19986	0·21894	0·18815	0·09518
Relative humidity	−0·05280	−0·14319	−0·18288	−0·18729
Rainfall	−0·15142	−0·16026	−0·12151	
Monthly mean temperature	−0·23637	−0·22892	−0·15335	

pressure figures with the March mortality from smallpox, and so on. The third set of figures shows the values for a delay of 2 months, and the last for one of 3 months.

Pressure.—This is the only climatic factor with a positive value, and the table shows that smallpox prevails when atmospheric pressure is highest. There is no significant difference in the figures for the first three lags.

Relative humidity.—The coefficients for all four lags are negative; that for lag_0 is not significant, and that for lag_3 being the highest, one can infer that a 3 months' lag occurs. The figures indicate that smallpox is not likely to assume an increased virulence when the relative humidity is high, such as is the case in the summer months.

Rainfall.—Here all values are significant and negative, with a lag of 1 month, showing that rainfall has a maximum inhibitory effect on smallpox after a delay of 1 month.

Mean Monthly Temperature.—Here again all values are negative and statistically significant, the maximum value being lag_0.

It can be argued that these figures tell one nothing more than what is known to anyone who has watched smallpox in the Far East, but the coefficients of correlation supply an exact value for Hongkong climatic values which enables the epidemiologist to compare them statistically with similar data in other countries.

(*f*) A COMPARISON OF THE COEFFICIENTS OF CORRELATION WITH WHAT HAS BEEN FOUND TO APPLY TO INDIA.

RUSSELL and SUNDARARAJAN (1929) gave separate figures for each of the thirteen divisions into which they divided India, so I cannot conveniently reproduce them, but it is easy to summarize briefly their results and compare them with mine.

Pressure.—These authors found that in India the coefficients were of much the same magnitude as for temperature, but of opposite sign. The same applies

to Hongkong. They found however that in India there is a 2 months' lag, whereas in Hongkong there is no lag.

Relative humidity.—Indian values were negative except in parts of Madras, and in most cases there was a lag of one month, all being statistically significant. In Hongkong, though all are negative, lag_0 is not significant, and there is a lag of 3 months.

Rainfall.—RUSSELL and SUNDARARAJAN found a negative association everywhere, with a lag of 2 months in most cases. In Hongkong there is a lag of 1 month, and it is also a negative association.

Temperature.—In India it was found that there was a 2 months' lag, the coefficients being negative and significant. In Hongkong also the values are negative and significant, but there is no lag.

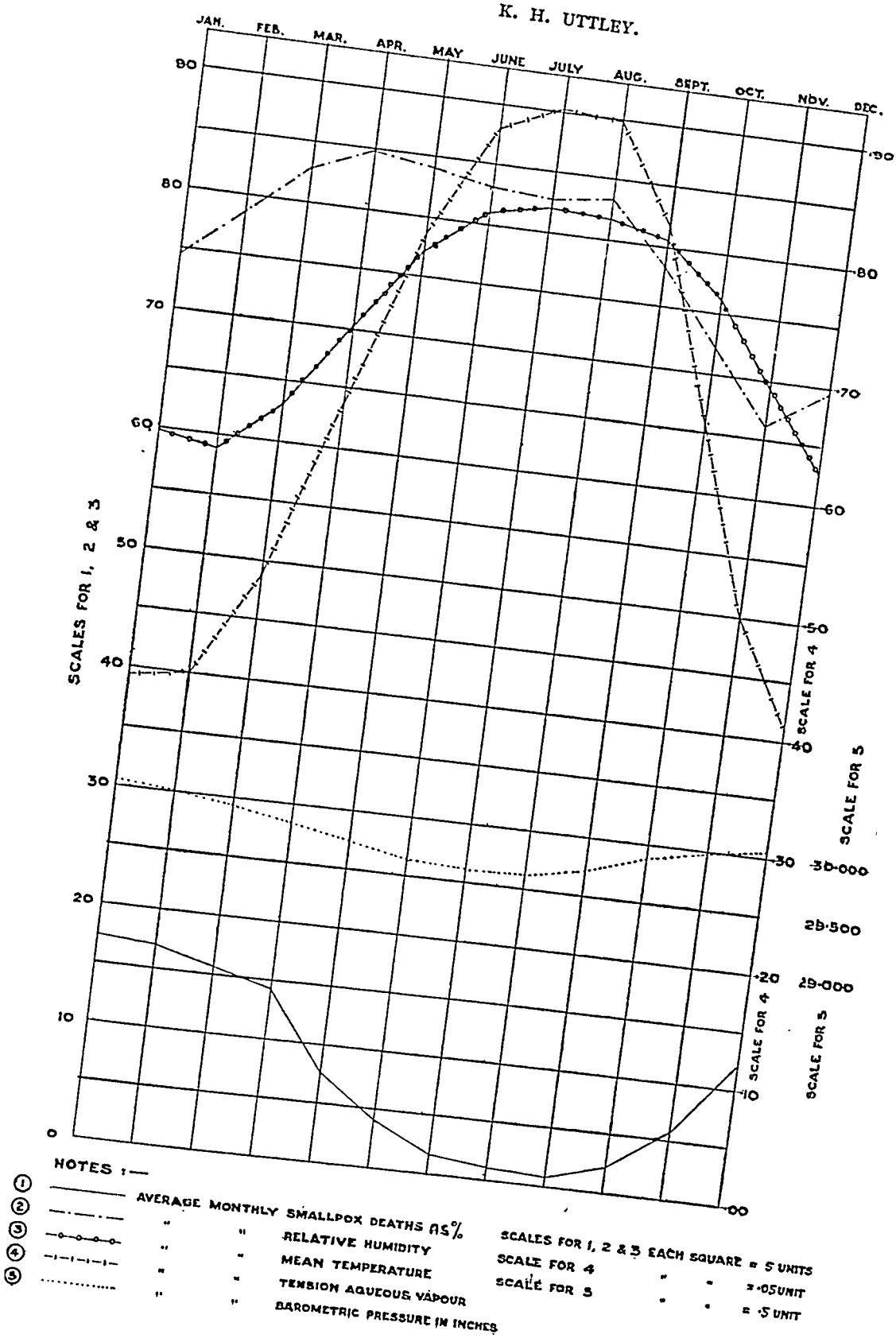
Climatic conditions in different parts of India vary considerably, but the comparisons I have made above are of interest when it is remembered that Hongkong is in the same latitude as Calcutta, and is subject to a wet monsoon in the summer and a cool dry season in the winter, as are many parts of India.

Table VI and the graph show the relationships between these climatic factors and smallpox mortality in another form. They emphasize what has already been said, namely that smallpox deaths occur mainly when pressure is high, and when all the other factors have low values.

TABLE VI.

RELATIONSHIP BETWEEN CERTAIN CLIMATIC FACTORS AND SMALLPOX MORTALITY.

Month.	Average monthly per cent. smallpox deaths.	Average monthly relative humidity.	Average monthly mean temperature.	Average monthly tension aqueous vapour.	Average monthly barometric pressure in inches.	Average monthly rainfall in inches.
Jan.	17·3	74·6	59·8	0·393	30·052	1·27
Feb.	17·0	78·6	58·9	0·400	30·013	1·75
Mar.	15·7	82·9	63·1	0·488	29·945	2·93
April	14·2	84·9	70·3	0·638	29·843	5·44
May	7·7	84·1	77·1	0·786	29·743	11·50
June	4·1	83·1	81·0	0·880	29·646	15·52
July	1·7	82·8	82·0	0·903	29·613	15·01
Aug.	1·1	83·4	81·7	0·899	29·616	14·22
Sep.	0·9	78·5	80·6	0·818	29·721	10·11
Oct.	2·3	72·2	76·2	0·657	29·879	4·55
Nov.	5·9	68·2	69·4	0·500	29·988	1·70
Dec.	12·1	69·5	62·9	0·410	30·047	1·15


JAN.
FEB.
MAR.
APR.
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
90
80
70
60
50
40
30
20
10
0
SCALES FOR 1, 2 & 3
SCALE FOR 4
SCALE FOR 5
36·000
29·500
29·000
NOTES :—
① AVERAGE MONTHLY SMALLPOX DEATHS AS %
② " " RELATIVE HUMIDITY
③ " " MEAN TEMPERATURE
④ " " TENSION AQUEOUS VAPOUR
⑤ " " BAROMETRIC PRESSURE IN INCHES
SCALES FOR 1, 2 & 3 EACH SQUARE = 5 UNITS
SCALE FOR 4 " " = ·05 UNIT
SCALE FOR 5 " " = ·5 UNIT

Conclusions and Summary.

In this paper I have made a survey of smallpox mortality among the urban Chinese of Hongkong during the last 40 years. Its crude death rate is very irregular, but over the whole period the mean is 0·46 per 1,000 living, the maximum rate being 2·27 in 1923, and the minimum 0·01 in 1931 and 1926. The standardized death rate, calculated for census years against the British population of 1901 shows that for most years the female mortality has exceeded that of males by three to two.

By calculating deaths at ages per 1,000 living, it is seen that infantile mortality is disproportionately higher than that obtaining in Britain, at any rate so far as the British figures for 1901 are concerned.

72 per cent. of all smallpox deaths have occurred in the first 5 years of life, and 53 per cent. have occurred in the first 3 years.

Until puberty there is a higher mortality among females, but thereafter males predominate.

The disease is a winter and spring disease, but sporadic cases occur at other times.

Vaccination, which had been employed on a very small scale previous to 1928, was in that year most intensively used, and has been so ever since, with the result that it has altered the percentage of total deaths from the disease occurring at different ages, mainly because small children form the majority of those vaccinated. The percentage of the total of smallpox deaths occurring under 5 years of age was 78 per cent. in the decade ending 1930, resembling the state of affairs in England in the eighteenth century, before the advent of vaccination. In the 6 years since 1930, when vaccination has been intensively practised in Hongkong, the percentage has fallen to 67·1.

It is likely, though not yet certain, that the low crude death rates met with since 1930 are in part due to vaccination.

I have attempted to carry out an investigation in Hongkong similar to that of Russell and Sundararajan in India on the role of the climatic factors of rainfall, relative humidity, temperature and pressure on the mortality from smallpox; and by calculating the coefficients of correlation for these factors and smallpox mortality, and comparing them with what they have found in India, I have shown that there is no very great difference in the results obtained in the two places.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

TROPICAL PHAGAEDENIC ULCER IN THE PACIFIC.

BY

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The observations here recorded are based on 892 cases of ulcer admitted to hospital during $8\frac{1}{2}$ years' work in three parts of the Southern Pacific. The cases were distributed as follows: Choiseul, Western Solomons, 110 cases;

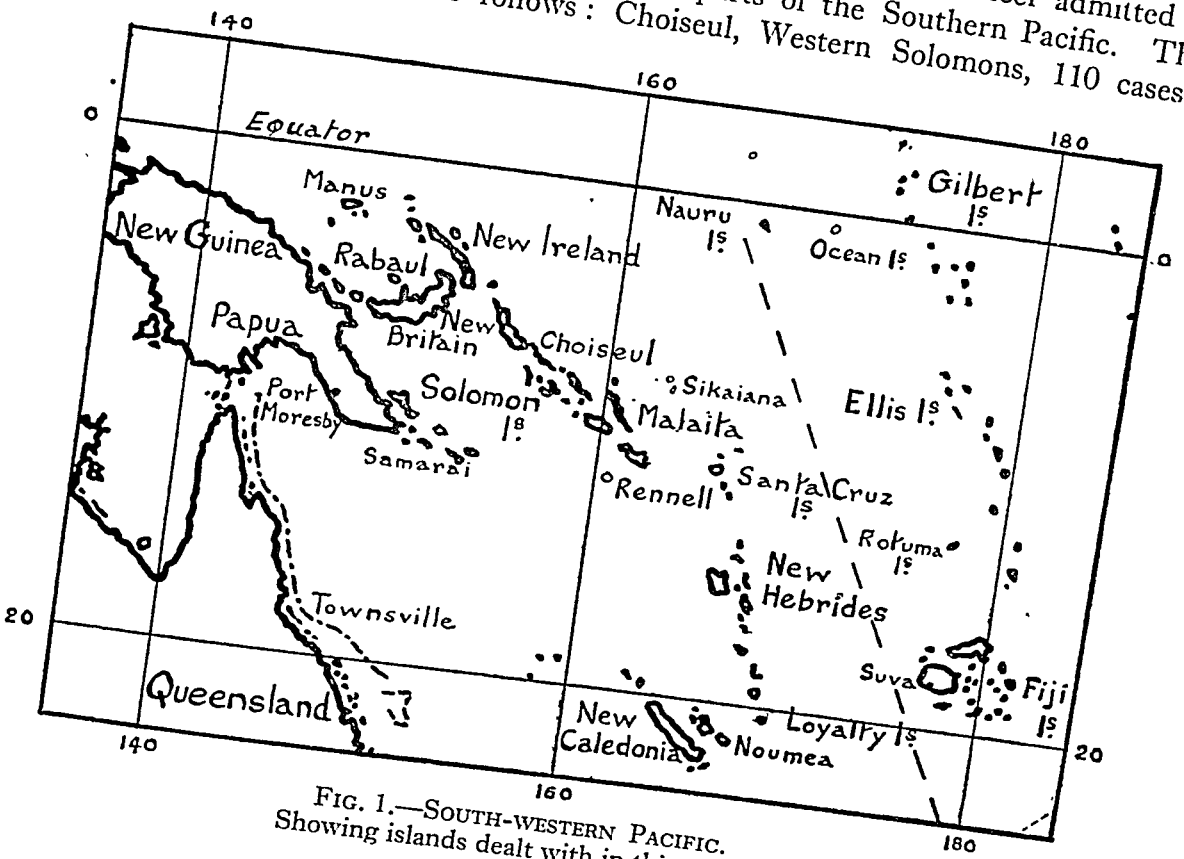


FIG. 1.—SOUTH-WESTERN PACIFIC.
Showing islands dealt with in this paper.

New Britain, Mandated Territory of New Guinea, 608 cases; Malaita, Central Solomons, 174 cases.

In addition to these there has been a much larger number of less serious cases treated as out-patients under my close supervision.

Tropical Phagaedenic Ulcer is due to an Infection.

(*a*) The whole aspect of an ulcer is that of an infection, especially in the more serious cases. High temperatures are common, e.g., 103° to 104° F. in children. A recurrence of the infection in an ulcer under treatment is first shown on the temperature chart. The pain will keep a child whimpering throughout the night. There is tenderness and swelling. Adenitis of the regional glands is not uncommon. The more acute the ulcer, the more marked are these signs.

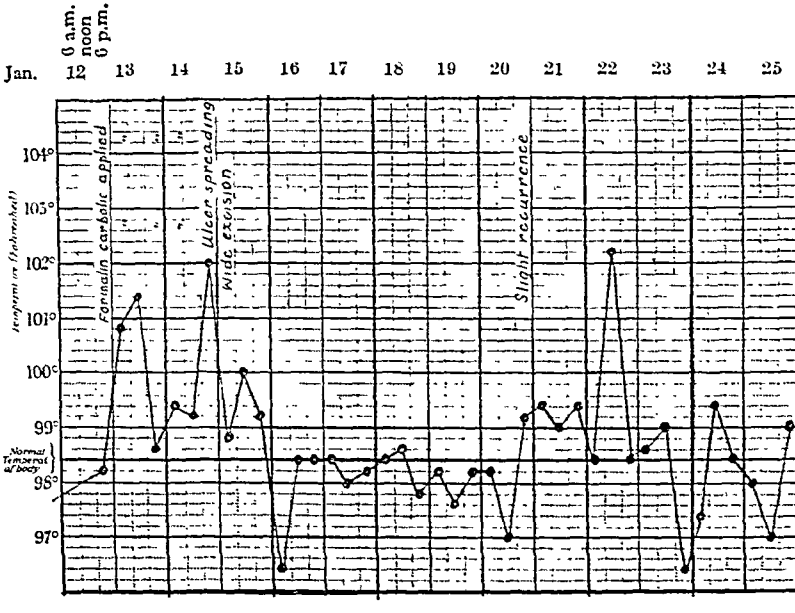


Fig. 2.—Chart showing relation of temperature to progress of ulcer.

Ia Mila, F., 4 months old, New Britain. Acute ulcer of whole of ball of foot. Pain, swelling, fever and toxaemia were marked. Caustics insufficient. Wide removal done (excision) and temperature dropped. On 20th, a slight recurrence of the infection (phagaedenic) with a rise in temperature. Caustics were applied, and ulcer became clean again. Skin grafts were applied, and patient discharged 12 days later.

(*b*) Complete surgical removal of an ulcer relieves these infective signs much as in a carbuncle. Similarly, in lesser cases, caustics applied to the ulcer will do the same.

Were the process, say, a tissue necrosis only such treatment would make things worse, not better.

(*c*) The discharges from a phagaedenic ulcer are actually infective.

Case : *Madeline*, 5 *years*, *New Britain*. Acute ulcer of the buttock (a serious ulcer in children). I excised it and was to skin-graft it later. It was dressed

with acriflavine and remained a clean slowly granulating area. Another ulcer developed on the mons veneris following her scratching herself. One morning I found that some discharge from the new ulcer had trickled down and had infected a triangular-shaped area of the clean granulations which now was an acute ulcer.

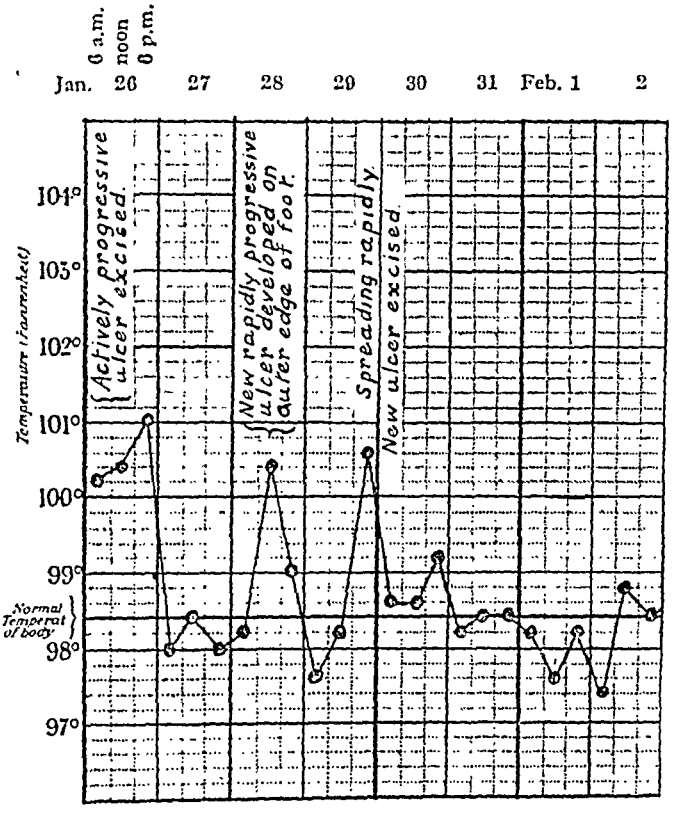


FIG. 3.—Relation of temperature to development and excision of ulcer.

Ia Pidikia, F., 29 years, New Britain. Acute ulcer, 5 days' duration, on inner part of sole of foot, 4 inches by 3 inches. Fetid, covered with a moist slough; and pain, swelling and toxaemia were marked. Wide excision followed by drop in temperature. On 2nd day, a new rapidly acute ulcer developed on outer side of foot. Temperature rose, and dropped again after surgical excision. After 13 days, grafts applied, and patient discharged 17 days later.

(*d*) The Cult of the " Immediate Iodine and Adhesive " will prevent any break in the skin from becoming an ulcer. I have never seen an ulcer develop where shoes and stockings have been worn and where any abrasion received a dab of iodine and was covered with a patch of adhesive plaster.

Bacteriology of Ulcers as Seen in Smears.

Clinically the ulcers which are met with may be classified as follows :—

Phagaedenic Ulcers.
- Active progressive ulcers.
- Subacute ulcers—where the progress has ceased.
- Clean, chronic or healing ulcers.

Non-phagaedenic Ulcers.
- Septic ulcers, etc.

There is a striking correlation between the bacteriological findings of the smear, and the clinical state of the ulcer. Whenever an ulcer had a definitely phagaedenic character fusiform bacilli were present.

Acute ulcers. All showed fusiform bacilli, while 75 per cent. showed spirochaetes and 65 per cent. showed filamentous forms.

Clean, chronic, and septic ulcers. These never show fusiform bacilli, spirochaetes or filamentous forms.

Sub-acute ulcers. These, as would be expected, occupy a midway position between the other two ; 82 per cent. showed fusiform bacilli, 50 per cent. showed spirochaetes, 31 per cent. showed filamentous forms.

Other bacteria seen in the smears were cocci and diphtheroids.

THE BACTERIOLOGICAL FINDINGS IN SMEARS OF ULCERS IN THE VARIOUS STAGES FROM THE " IDIOPATHIC " ULCER TO THE HEALING ULCER.

Stage 1.—*The " idiopathic " ulcer.*

I had six natives who came for treatment for a condition, usually a vesicle, which they definitely considered would become a bad ulcer if untreated. The lesions were all unbroken, were painful, and there was no obvious cause for their appearance. The contents were a dirty greyish fluid, and the floor was moist and greyish. Smears showed Gram-positive cocci in diplococcal form. Some time previously I had inoculated similar material into broth, and typical staphylococci grew from it, but in smears from lesions, typical "bunch of grapes" formation is rarely seen. The ordinary boil shows only cocci in diplococcal form (Stitt, 1927).

All the above were treated, mostly by a dab of caustic (carbolic) and all healed without any phagaedenic infection occurring.

Stage 2.—*Lesions of the above type just after the vesicle had broken.*

Jope, M., 20 years, New Britain. Acute ulcer on one leg. Later a vesicle developed elsewhere on the same leg. It had broken some little time before I saw it. The discharge contained a few fusiform bacilli as well as cocci. This was evidently a secondary infection just starting.

Malachi, M., 22 years, Solomons, also had a vesicle which had burst and the surface was only partly covered with the epithelium. A smear similarly showed cocci and some very fine fusiform bacilli,

Stage 3.—*The fully developed phagaedenic ulcer.*

Ilias, M., 20 years, New Britain. A blister similar to the above had broken 24 hours before. The surface was darker, obviously phagaedenic, painful and a little swollen. A smear showed fusiform bacilli, spirochaetes, and filamentous forms.

Stage 4.—*Cessation of the phagaedenic process.*

The spirochaetes and filamentous forms disappear first, the fusiform bacilli become shorter, and then disappear.

Stage 5.—*A possible recurrence in a clean ulcer.*

This happening is not uncommon, the smear then showing a re-appearance of the phagaedenic germs. *Damiri* showed first fusiform bacilli and filamentous forms. After the recurrence, there were fusiform bacilli and spirochaetes.

The Smear of Certain Types of Ulcer is fairly Constant.

(*a*) A messy, fetid, relatively superficial ulcer usually shows both fusiform bacilli and spirochaetes.

(*b*) The particularly chronic ulcer of the toe-nail area shows a similar picture.

(*c*) An ulcer with dark red granulation tissue from which can be expressed a thin pale pus shows usually fusiform bacilli only.

(*d*) Ulcers with more or less deep subcutaneous extensions show fusiform bacilli, but no spirochaetes. Such ulcers are :—

1. A deep ulcer with raised edge from which pus can be expressed.

2. A progressive ulcer with long finger-like extensions, the extremities of which break down and form new ulcers. These join with and enlarge the parent ulcer. Healing is by scar tissue in the centre, spread being centrifugal.

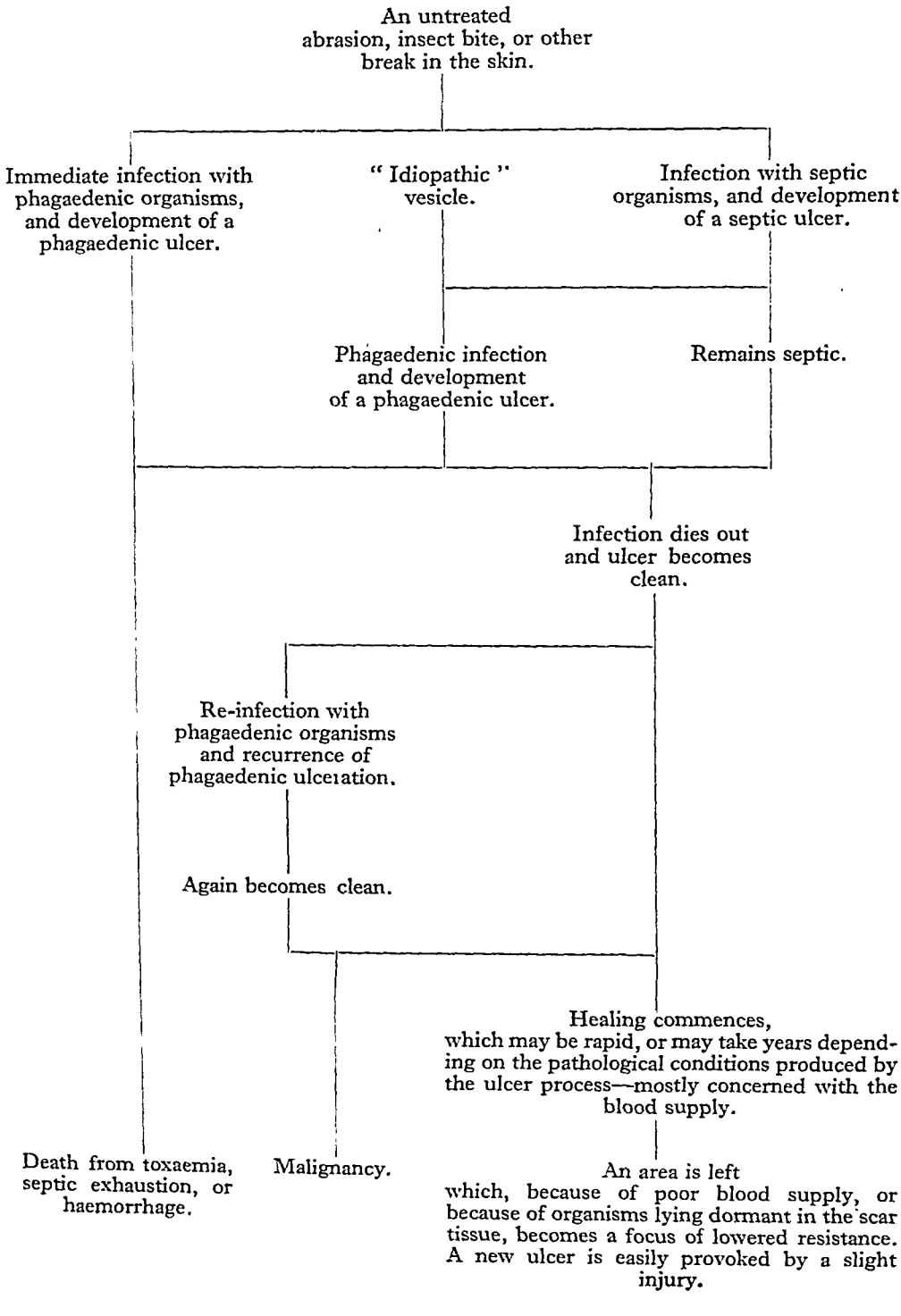
3. More serious types with extensive spread along fascial planes, and subsequent huge sloughs, the skin being intact until the last.

The microscopic picture of types 2 and 3 often resembles that of SMITH's experimental ulcer in the hedgehog (SMITH, 1936, Fig. 3).

The fusiform bacillus ulcer is a less rapid one, the spirochaetes seeming to add more virulence to the process, especially superficially.

The filamentous forms may be long and rather thick and may be seen dividing to form fusiform bacilli, or they may be finer and show every gradation between a long straight form and a definite spirochaete. Some filamentous forms are exceedingly fine. Some smears seem to show every step between the common fusiform bacillus and the spirochaete via the filamentous forms, as though they were all actually one organism.

THE SEQUENCE OF EVENTS IN THE PATHOLOGY OF AN ULCER.



Clinical Notes.

GENERAL DESCRIPTION.

Ulcers are too common to need much description. In serious cases, they may increase in diameter one finger's breadth in a night. Toxaemia seems to prevent a marked local inflammatory reaction. A vicious circle may be established, the toxaemia enabling the ulcer to increase in size and so to increase the toxaemia.

Where the ulcers are of the bad type, as for instance, in New Britain, the average age of the patient is less than in other places (12 years as compared with 23 years in the Solomons). In New Britain, one-third of the cases were in children under 8 years of age, and 2 per cent. occurred in infants under 1 year.

In the last-named place, ulcers and their complications (toxaemia, deep sepsis, exhaustion) were the chief cause of death in the hospital. To save life, urgent amputations and blood transfusions were often necessary, though amputation may be risky because of extensive subcutaneous extensions of the phagaedenic process.

A healed ulcer is certainly better than an open one, but nevertheless it remains a liability to its owner. Scar tissue is formed in varying amounts in healing. It is most abundant in slow healing, and its disadvantages are :—

(*a*) It diminishes the blood supply to the new skin.

(*b*) It contains, in its interstices, dormant organisms. Smith (1936) confirms this in finding numerous organisms in the base of an almost healed ulcer (experimental) in the hedgehog.

(*c*) The blood vessels in the ulcer area suffer with an obliterative endarteritis (Smith, 1932) which narrows the lumen and cuts down the blood supply.

The result of this is that the scar tissue is liable to break down with the formation of a new ulcer, destroying the work of weeks of dressing. If surgical interference is necessary to bring about healing, as much as possible of the scar tissue should be removed. It is often amazing the amount that has to be cut away before normal tissue is reached.

Malignancy has occurred once only in my experience, the case being an ulcer of the shin of a lifetime's duration in a man of 40 years of age. The malignant growth was from the bone in the base of the ulcer. Vint (1935) in Kenya has noted malignant changes in 2 per cent. of a routine unselected series of ulcers excised for treatment. Whatever may be the actual frequency of this condition, its occurrence points to excision as the best treatment for these ulcers.

The ordinary common septic ulcer in the tropics, without any phagaedenic infection, is surprisingly slow to heal. The humidity and the increased sweating make the skin sodden, so that a good medium for the profuse bacterial life of the tropics is provided. This militates against quick healing while incidentally dressings are difficult to keep on.

The part played by Defective Nutrition in the Causation of Ulcers.

The influence of diet on the progress of the ulcer has been impressed upon me by such events as the following :—

(*A*) A continued activity or recurrence of the phagaedenic process in certain cases for no obvious reason mystified me until I enquired about the diet. This was found to be inadequate, and correction of the deficiency changed the aspect of the ulcer.

Case 1.—*Komai*, M., 22 years, New Britain. A nicely healing ulcer suddenly became phagaedenic, and subsequently a clean excision and skin-graft did the same. It was later found that he was living on two cooked green bananas per day. Increase in diet (rice) caused healing.

Case 2.—*Takana*, F., 14 years, Malaita. A clean excision and skin graft suddenly became phagaedenic. It was found that she did not like the rice and biscuits of the hospital diet. Native food was secured and the ulcer healed.

Case 3.—*Tapliu*, F., 13 years, Malaita. Developed an acute ulcer while in hospital on routine quinine, iron and cod liver oil, but, as in the above case, she would not eat the hospital diet. On a change of diet, the ulcers healed.

Case 4.—*Madeline*, F., 5 years, New Britain. Continued to develop new ulcers while in hospital. Her food was found to be quite insufficient. She ate (rice) ravenously and her ulcers healed.

These four cases had all lost weight as a result of their deficient diet which was at fault as regards quantity rather than quality.

(*B*) The occurrence, for no obvious reason, of one serious ulcer in a village of healthy people.

Case 1.—*Dina*, F., 3 years, New Britain. An ulcer developed upon the site of an old healed burn following the patient's scratching it. After only 6 days, it measured 3 × 4½ inches. Toxaemia was marked and with it were a high temperature and a poor pulse. Deep extensions along tendons and between muscles demanded amputation and two blood transfusions to save her life. It was discovered later that the family were in difficulties with their garden, and were often hungry. Both the mother and child were under-nourished. A poor appetite from the toxaemia cut down still further the child's food intake, and thus a vicious circle was established.

(*C*) Epidemics localized to a single community while surrounding villages were not affected.

1.—From the 7th March to the 3rd April, the village of Vunavulila, New Britain, supplied me with a group of 12 ulcers. Of any such groups of ulcers, which I have met, this was far and away the worst. All were acutely phagaedenic, and needed wide excisions, and in two cases, amputation.

In February this village, with a population of about 120, had held the great feast of its lifetime. Long self-denial and economy in food provided the pigs and food which were distributed amongst the guests, and demonstrated the affluence of the community. For 2 or 3 days the people enjoyed themselves to the full. Then came a time of empty gardens and little to eat. Six weeks later, 12 of the villagers were in hospital with serious ulcers and others had less serious ones.

It is difficult not to blame the sudden lack of food as being a predisposing cause of the ulcers.

2.—In New Britain, the hospital was situated on a station consisting of a girls' school and a boys' school. In September the headmistress of the former left and was relieved by one who did not understand the gardens nor the feeding of a large number. The gardens

were emptied and no new ones were planted. From Christmas until the return of the original mistress in April, the girls were hungry.

The graph below shows the admission rate to hospital for ulcers.

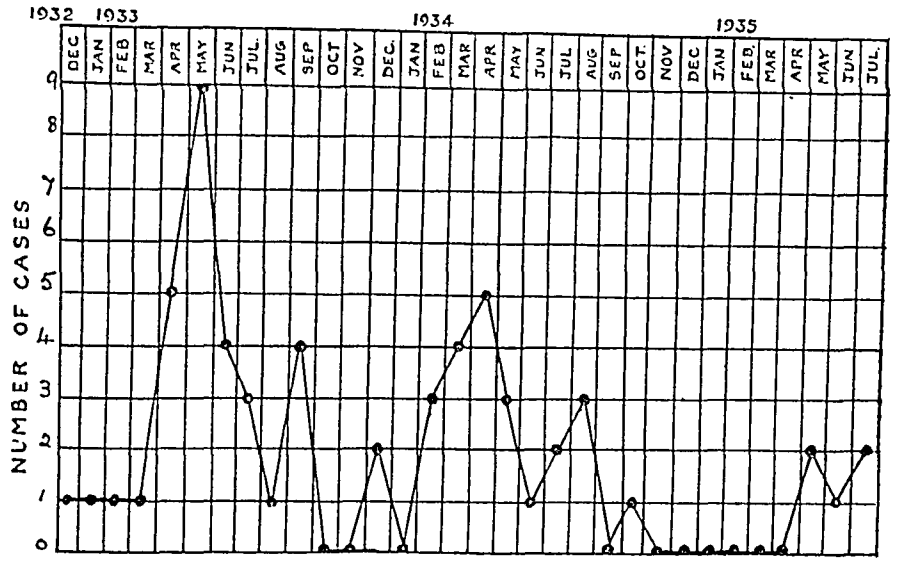


FIG. 4.—Girls' school—admission rate for ulcers.

3.—Similarly the boys' school suffered a change of headmasters in December. The reserve food in the bush gardens had been stolen, and during the development of new gardens there was a lean and hungry time for the boys. Rice had to be bought to increase the food supply.

The graph shows the admission rate of the boys to hospital with phagaedenic ulcers.

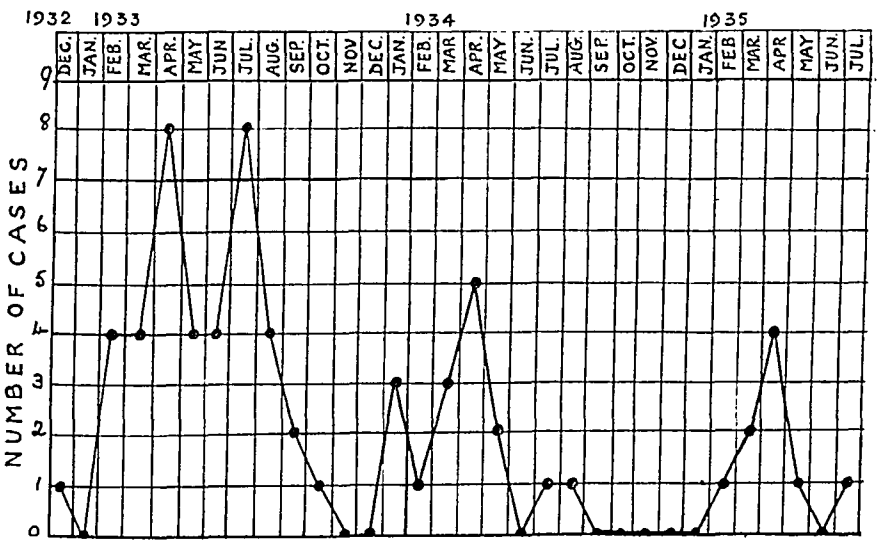


FIG. 5.—Boys' school—admission rate for ulcers.

The above students were under my care for 3 years. They were isolated, and their living conditions were more or less constant till the shortage of food described above occurred. That it should occur in the two schools and be

of the same type and that it should be associated in both schools with a rise in ulcer rate is very suggestive of a relation between the food and the ulcers.

(*D*) The variation in the type and incidence of ulcer in New Britain, Western Solomons and the Central Solomons.

In certain characteristics these three places resemble one another. Thus :—

1. All possess hot moist tropical climates and range in latitude from 4° to 8° south. They are mountainous and bush-clad.

2. The natives are all pure Melanesians and the variety of their food is more or less the same.

3. Malaria and phagaedenic ulcers are universal throughout.

The differences from the point of view of this paper are to be found in the amount and quality of food eaten, and in the incidence and severity of the phagaedenic ulcers.

Choiseul and Malaita (British Solomons) are both islands over 100 miles long. They are more mountainous than New Britain, and with their numerous rivers there are swamps which breed mosquitoes in abundance.

The *Gazelle Peninsula* is the north end of New Britain. It is hilly and thickly populated and contains Rabaul, the capital of the Territory. Not all the land is good garden land, and much of it will not grow taro, the staple food. Where natives elsewhere will eat taro, many in these parts eat poor quality potatoes (native) which will grow on poorer soil. As well as supplying their own wants, the population caters for Rabaul's wants, which are extensive. Rabaul's white population is 500 and its " Chinatown " has 100 merchants : with these there is the large population of native labourers, and the natives employed by the Government. Many ships call and are supplied from the natives' produce. The big native market collects food from far out in the bush and includes all the area under consideration. Natives grow their own foods for this market, and also such things as tomatoes, cabbages, beans, etc., for sale to white people. The natives will sell anything ! If they catch a fish, they hold it up for sale at the roadside. They rear fowls for sale, and they sell their cocoanuts which on other islands are used as a reserve food. A large part of the population has no access to the beach for fishing purposes. The result is, in my opinion, that the people are underfed, and poor food and not much of it is the rule.

The picture presented by the Solomons is very different. Here there is comparatively good food and plenty of it. Similarly ulcers were the chief cause of mortality in New Britain while in the Solomons there has been only one death in $4\frac{1}{2}$ years. The seriousness of the disease in New Britain is due, I believe, to the poor state of nutrition of its inhabitants.

(*E*) The definite correlation between the frequency of phagaedenic ulcers and the season of the year.

Generally speaking, February to July are the " ulcer months," with a peak in April. Ulcers are uncommon in the latter part of the year and during the month of January.

The north-west season (monsoon) starts sometime between the end of November and the end of December, and continues until April. It is a season of irregular weather, hot dry spells alternating with periods of drenching rain and gales. The heat and dryness kill the gardens, and in the wet the vegetables rot in the ground. The gales blow down the trees (banana, etc.). Where the gardens are at a distance the people cannot visit them. Fishing is impossible.

The height of the "ulcer period" corresponds to the time when the people will be feeling the effects of the previous months of precarious food supply, the gardens will be at their poorest, and the bodily reserves will have been used up. In 1937, the north-west season was delayed, the first storm occurring in the middle of January. There was a corresponding delay in the "ulcer period."

THE ORDINARY FOOD HABITS OF THE NATIVES MUST TEND TO MALNUTRITION.

(*a*) The natives are, of necessity, vegetarians. Fish is an "extra," except for those living on the beach.

(*b*) They bolt great mouthfuls of insufficiently chewed food. The appearance of the contents of the bedpans is similar to that of their plates before the meal.

(*c*) On long tramps they do not take food with them: they chew betel-nuts which assuage hunger but have no food value.

(*d*) They have one proper meal per day—in the evening. If there is any left over, it is eaten for breakfast, if not, there is no meal in the morning. They may chew a piece of cocoanut.

(*e*) In the nutting season, they live in the bush on nuts—a tough, indigestible native almond—which are quite insufficiently chewed. Their stomachs are full, but actually they are starving.

(*f*) They care little about the future. They eat while there is plenty and will eat too much, and waste food, but they do not put any away for the morrow. A little counter-attraction, and they will forget all about their gardens which rapidly deteriorate.

(*g*) Gardening methods are very crude, no manuring is done, and wild pigs raid the gardens unless they are fenced. Bad weather, sickness, when no work can be done, and feasts, when the gardens are depleted, all take their toll from the native's menu.

WHAT IS THE VITAL ELEMENT IN THE FOOD WHICH IS LACKING?

In hospital, the routine medicinal treatment for all ulcers has been quinine, iron in fairly big doses, cod liver oil, marmite as a morning drink when fresh vegetables are insufficient or in doses of 12 grammes per day for experimental purposes.

Vitamins are supplied in titbits of greens and fruits.

Calcium is supplied in abundance in the betel-nut chewed.

Food arrangements: Choiseul—plenty of native food. New Britain—native food (not plenty) supplemented with rice. Malaita—rice and biscuits and native food when possible.

Progress of seven illustrative cases on above regime :—

1.—Ulcer progressed. Amputation became necessary.
2.—A recurrence of phagaedenic infection after 3 weeks.
3.—A new ulcer developed after 4 weeks.
4 —Ulcers continued to develop after some weeks.
5.—New ulcer developed after 17 days.
6.—New ulcer developed after 20 days.
7.—A new phagaedenic infection developed after 20 days.

Other similar cases also suggest that ulcers will develop while a sufficiency of the diet described above is being taken.

One is driven to the conclusion that there must be an insufficiency of suitable protein. On the other hand, it is questionable whether it is possible to point to any one item as being the only deficient factor. If it is protein which is chiefly lacking there may be also a shortage of other substances which prevents the body from making good the deficiency in protein. This would happen when there is a general all-round insufficient intake, and would explain all the foregoing problems (*A* to *E* pp. 654 and 656). In village life a good supply of native food will supply the protein, or a lesser supply of food containing a better protein, e.g., fish. In the following two places much fish is eaten, and though the total food is not great, ulcers are rare.

1. *Sikaiana* is an atoll, 10 miles long. The amount of land suitable for gardens is small, but the lack of vegetables is made up by a splendid fishing reef and lagoon. The natives delight in fish. The population is increasing in spite of a good deal of malaria. The diet is coconuts, taro, yams, a few fruits and fish.

2. *The Artificial Islands* of the north-west lagoon of this island (Sikaiana) are most interesting. Throughout the area of the lagoon (30 miles long, and 1 to 2 miles in width), there are scattered thirty islands built up from the surface of the reef, at enormous labour by lumps of coral carried from elsewhere on the reef. Lying just above highwater mark, the islands have big populations. On one, 300 people live on an area less than an acre. There are crowds of children who are clean, non-malarious, but rather thin. The inhabitants say they do not have ulcers, but after visiting a number of them, I found one small phagaedenic ulcer on an islander, and two other ulcers with a yaws basis. This was in the " ulcer season."

The food was mainly fish from the reef, but some vegetables are secured by barter from the mainland. The infrequency of ulcers is remarkable.

In both places, fish in the diet evidently not only makes up for the lack of plenty of food generally, but supplies good protein as well.

Relationship between Malaria and Phagaedenic Ulcer.

That such a relationship exists is suggested by the following :—

(*a*) White people suffer with phagaedenic ulcers in Melanesia, but it is my experience that such ulcers do not occur in non-malarious white persons. The cases seen by me either took no quinine or took it so irregularly that they suffered attacks of malaria. It may be noted here that all who have given prophylactic quinine a thorough trial know that it is possible to go for years without suffering a malarial attack, and, if the quinine is taken properly, it will not produce any of the adverse symptoms which are recorded in the literature.

I can recall nine patients who consulted me for phagaedenic ulcers, and they all had malarial attacks with varying frequency.

(*b*) I have been consulted by eight white people who were non-malarious, took their quinine regularly, and had ulcers, but all were simple, septic non-phagaedenic ulcers.

Two cases have impressed me :—

1.—" M " did not know the efficacy of quinine in malarial prevention. She had malaria frequently and developed two serious phagaedenic ulcers which needed one month in bed and another month sitting about the house before they were healed. During her next term of service, she took quinine most regularly, and though she continued to go into the gardens without stockings, and though she had less fresh meat and butter than before, she was entirely free from ulcers for at least 3 years.

2.—The case of a medical colleague who did not believe in quinine at first, and had frequent malaria. He developed a phagaedenic ulcer which troubled him considerably.

I myself, am a strong advocate of quinine, am non-malarious, and have never had an ulcer of any sort during $8\frac{1}{2}$ years in the tropics.

(*c*) On the map p. 647 at the commencement of this paper, I have drawn an interrupted line. To the east of this line, there are no phagaedenic ulcers, but to the west, they are common. I was in Fiji recently, and Dr. Clunie of the War Memorial Hospital very kindly showed me two cases of tropical ulcer as it occurs in Fiji. Both were on the foot, about $1\frac{1}{2} \times \frac{3}{4}$ inches in size, were not very deep, but were moist and a thin, pale discharge came from them. The base was of pink granulations, and the parts were a little congested. A smear showed no phagaedenic organisms, merely cocci and diphtheroids being present. There was no offensive odour. Clunie and Eva (1934) state that these ulcers occur in epidemics in schools, in road and other labour gangs. They suggest a dietetic cause, white bread and tapioca being largely the diet. The blood calcium was normal. The ulcer commences at the site of an abrasion. Strapping was the best treatment.

Accustomed to phagaedenic ulcers, I realized that these were tropical ulcers without the phagaedenic infection. The ulcers of my experience were definitely the dietetic ulcers of Fiji, with a superimposed phagaedenic infection which did the damage. My " idiopathic " ulcers were miniature Fijian ulcers, the cause probably dietetic, and the cocci the same.

The freedom of Polynesia from phagaedenic ulcers is not a natural or racial immunity. Fijians are a mixture of Melanesian and Polynesian strains. Polynesians in Melanesia are not immune to ulcers, while Melanesians from the Solomon Islands do not have ulcers when they go to live in Fiji.

What is more surprising still is that the above-mentioned line on the map not only separates the " phagaedenic west " from the " non-phagaedenic east," but also separates the malarial west from the malarial east. Other diseases are the same on both sides of the line. (The Indian immigrants to Fiji have added some diseases in recent years, but these do not alter the general statement).

It appears, therefore, that when a native to the west has a dietetic deficiency, because of his inevitable malaria, he suffers from phagaedenic ulcer. On the other hand, to the east, a native with a similar deficiency, is liable to non-phagaedenic ulcers.

(*d*) The spleen of the average ulcer patient is larger than that of the average general population, by an amount equal to one-third of the distance between costal margin and umbilicus.

(*e*) *Rennell* is an atoll which has been thrust up 300 to 400 feet from the sea. It is 50 miles long, and lies 90 miles from the nearest big island. Inland there is a lake and elsewhere the land is fertile in spots. The interest of Rennell is that it is peopled by Polynesians (amongst Melanesians on all the other islands). The food is yams, taro and pandannus fruit ; spinach, coconuts, pawpaws, fish and shell fish, are eaten at times. Unlike Melanesians, they have no meat hunger, and will not exert themselves to catch the birds on the lake. Their canoes are too frail to go out to sea. LAMBERT says, " They live near the vitamin and food life-line."

Though Rennell is in the malarial belt, there is no record of any malaria in the past. LAMBERT visited the island in 1930 and 1933 (LAMBERT, 1932 and 1934) and found *no malaria, no anopheles mosquitoes,* and *no phagaedenic ulcers.* It was probably populated from a non-malarious people at the start, and its isolation has protected it since. He reports the general health as being excellent.

With LAMBERT'S account may be compared the report of Dr. CRITCHLOW, of the Government Medical Service (Dr. H. B. HETHERINGTON, S.M.O., kindly allows me to quote the figures). He visited the island in August, 1936. In two districts with a population of 126, he examined sixty-eight natives, and found twenty-five phagaedenic ulcers, five cases of malarial fever, and seventeen enlarged spleens (malarial).

He did not examine all the people so that it seems safe to conclude that there were most probably other cases of enlarged spleen, and possibly ulcers.

The natives told Dr. CRITCHLOW that three Rennell islanders, after returning from other islands, developed phagaedenic ulcers, and that the disease spread and caused many deaths. In some cases the spleen was a hand's breadth below the costal margin. Anopheles also had been introduced. It thus appears that in 1930 and 1933, there were no ulcers, no malaria, and no anopheles on Rennell,

and that between 1933 and 1936, all three were introduced so that in 1936, there was at least an incidence of 20 per cent. ulcers and, say, 20 per cent. malaria.

Because of the serious state of things, the island has been proclaimed a " closed " territory and a medical worker put there.

The fact that both malaria and ulcers have gained a hold is significant, because the natives say that, in 1911, there was an epidemic of ulcers in the island but that it died out. No malaria or anopheles were introduced. Now ulcers are well established, because, in my opinion, malaria favours their development.

(*f*) Malaria causes destruction of the blood. The average haemoglobin values of ulcer patients were as follows : active progressive ulcers, 66 per cent. ; sub-acute, non-active ulcers, 70 per cent. ; clean ulcers, 76 per cent.

The haemoglobin value of the general population was 75 to 85 per cent.

If the haemaglobin value is taken as a rough gauge of the activity and seriousness of the malaria (clinical hookworm is a rare condition here) the above figures would suggest that the more active the malaria the more liable the patient would be to develop an ulcer. Anaemia alone, however, is not the essential factor for cases of anaemia much below the above level may not have ulcers. It is the more chronic cases which have ulcers. (It has already been pointed out that the spleen rate is increased in ulcer cases.)

In malaria, there is a definite toxic factor, hence it is the chronic malarial native, saturated with toxins produced in his big spleen, who develops ulcers. This specific poisoning may lay the body open to the phagaedenic infection, a supposition which would explain the absence of ulcers in Fiji, because it can hardly be imagined that the phagaedenic bacteria have never reached this island.

A further point is that malaria with its fever means a constant wastage of valuable food materials from the body, the destruction of protein being very great (PEMBREY, 1933). Thus the good effects of a balanced diet are largely lost quite apart from the loss of appetite which cuts down the intake of food.

PREVENTION AND TREATMENT OF PHAGAEDENIC ULCERS.

Prophylaxis consists in the prevention of infection by wearing shoes and stockings, and applying iodine (immediately) and adhesive to any break in the skin. To these must be added a properly balanced diet and the avoidance of malaria—if contracted the malaria must be treated.

Curative measures involve the eradication of the infection, the encouragement of epithelialization, the prevention of recurrence and the treatment of malaria and dietary deficiency.

The first procedure is to apply to the ulcer a caustic such as a modification of that recommended by MCGUIRE (1934). Grind up as much copper sulphate in glycerine as it will absorb, and to the resulting solution, add to each ounce 1 dram pure carbolic acid as an anaesthetic.

This solution is applied daily to bad ulcers until the surface feels hard and granular. If there is pain or swelling, apply a small dressing of acriflavine (1/1,000) and cover it with a hot fomentation and protective (baked banana leaf is good), twice daily. Continue until the ulcer is clean. The above solution cannot be too thoroughly applied.*

When the ulcer is clean, if it is small, apply scarlet red ointment and adhesive plaster or elastoplast. (This fits any part of the body, but is not impervious, and infection can penetrate it.) Leave on for a week or more. Catastrophes will occur if adhesive is applied to ulcers which are still active. Absence of phagaedenic bacteria is a good test for loss of activity.

For larger ulcers, I am convinced of the value of excision and immediate skin-graft. This is usually easy because there is swelling which elevates the ulcer above skin level. It is removed in one slice, and seed grafts are put on at once to the raw area. Even with chronic ulcers, a little care will remove the ulcer in one piece. If too widespread for complete removal in one piece, the area must be considered infected and should be painted over lightly with pure carbolic (i.e., after removal of all the infected tissue). It is then clean. After 3 or 4 days, if still clean, it can be grafted.

Dressings on clean areas or grafts need not be changed for a week or 10 days unless the odour or temperature chart suggests infection. The experienced nose soon distinguishes between harmless decomposing blood and true phagaedenic infection.

In an earlier paper (JAMES, 1932) I have described the procedure in which over a number of cases, healing averaged 13 days. Since then, I have continued the method, with a few modifications, and the total figures are :—

	Number of Cases
Excision of ulcer with immediate skin graft	176
Excision of ulcer with delayed graft (serious cases with doubt as to complete eradication of infection)..	117
Excision followed by suture	20
Grafts applied to granulation surface without previous excision ..	19
Curettage of granulations followed by graft	8
Total	340

For success in these operations, I would stress the following :—

1. In long-standing ulcers, after removing the ulcer, the scar-tissue should be whittled away until as nearly as possible normal tissue is reached. The

* In preparing fresh solutions, care must be taken to wash out all crystals from the bottle to prevent the crystallizing-out of the copper sulphate.

brous tissue extends to amazing depths, and the less of this tissue there is, the ess chance there is of recurrence, the more knocks the new skin will stand, the ealthier the skin and the better the blood supply.

2. Granulation tissue forms fibrous tissue wherever it may be. This ontracts and cuts down the blood supply, and a successful graft may later ecome wrinkled and less resistent. Grafts applied to such granulating surfaces hould be put on when there is the thinnest possible layer of granulations, i.e., t the earliest possible moment, otherwise the granulations should be excised and ıe grafts applied to a bed of normal tissue.

3. In bad ulcers, the removal must be wide, knowing that any diseased ssue remaining will surely slough. A follow-up of my big excisions shows urprisingly good results. After removal of the tendo Achilles in one case, ıe man could, some months later, stand on the toes of that foot with the other aised from the ground. The posterior tibials and peronei had taken over the ork. If tendons remain covered by their sheaths, they will probably survive, ut if bared, they either slough or are involved in granulation tissue.

4. In fleshy parts such as the buttock, a " V " shaped incision allows the ides to be sutured together like a wound.

5. For the obstinate ulcers of the toe-nail area, the bone is often involved. y splitting the toe down so that the ulcer and bone are in one-half, and the pulp ı the other, a cross-cut can be made just proximal to the nail-bed. This will ut skin and bone and will meet the " splitting " incision. Sutures are put in as or an amputation. There is surprisingly little shortening, no loss of function, nd I believe the toe lengthens a little afterwards.

6. In the slowly healing ulcers of the sole, excision and grafting are good and ealing rapid.

7. Grafts applied over a hard, bony surface often do quite well, there is no sepsis. The skin, however, will never stand rough treatment, and bandage should be worn permanently.

8. In very chronic ulcers, over a bony surface, with the bone usually nlarged, the blood supply is never sufficient for a graft to do well. The equent protuberance of bone is not due to thickening of the cortex of the bone. have had good results in ulcers of a lifetime's duration by first excising the ulcer, ıen chipping away the bone carefully, and in several places over the area, ıaking an opening into the medulla about the size of the end of a pencil. The lood supply of the medulla is good, and granulations grow out from these holes ver the bare bone, and in a few days there is a surface of good granulations on hich to put the grafts.

In grafting, I use " seed-grafts " obtained by lifting up a tent of skin with needle and cutting this tent off just below the needle with a safety razor blade. s long as there is a bluish tinge in the little hole left, it is of right depth. These

seeds will adhere to an oozing surface—in fact, they help to stop any oozé—and also they will stand a certain amount of sepsis without dying. Their " survival rate " is high. If oozing does persist, put the dressing on firmly and, in bed, raise the leg to a right angle : the ooze invariably stops. A tourniquet is a help during the larger excisions.

Pre-operative preparation need not be done until the patient is under the anaesthetic. Soap well, shave, soap again, rub over with a methylated spirit swab and then with sterile water. Grafts are taken from the thigh on the handiest side.

Ordinary boiled mosquito netting, or better " parawax," is placed over the grafts, and a lotion of equal parts glycerin and red lotion is used on the wool. After a week or 10 days in order to remove the dressing, remove as much of the wool as possible, apply a thick pad of wool soaked in eusol and cover with some protective. The dressings will come off easily in a few hours. Scarlet red and adhesive or eusol or zinc ointment may then be put on until all is healed over.

Plaster of Paris is often of great help, especially if the lesion is over a joint or muscle. It also keeps the patient in bed.

As regards anaesthesia—I have been using the spinal form for over 8 years and am a firm advocate of it. With practice, it is easy, reliable and non-dangerous. No anaesthetist is needed. If there should be a drop in blood pressure—most rarely—shown by sighing respiration or restlessness, simply lowering the patient's head is all that is necessary. There have been no mishaps of any moment in over 500 " single-handed " spinal anaesthetics. I use percaine (Ciba) 1/200 solution with 6 per cent. glucose added to make it a heavy solution. By arching the patient's back with a pillow under the buttocks, and two under the shoulders and head, the solution can be localized. The average adult dose is 1·6 to 1·8 c.c. injected between the 3rd and 4th lumbar spines. A child of 10 years would take about 1 c.c. Younger children need a " whiff " of chloroform to keep them quiet during the injection, otherwise their crying sends the anaesthetic out through the puncture hole by the increase in pressure in the spinal fluid. I have used the method in a child of 1 year quite successfully.

For applying seeds to the granulation-surface of a clean ulcer, an infiltration of novocaine under the skin of the thigh suffices.

For finger and toe operations, an infiltration around the base is all that is needed.

Non-specific protein therapy with omnadin (Bayer) or edwenil, is of great assistance where the patient's vitality is doubtful. It certainly helps the grafts to " take."

In one difficult case of 20 years duration, arterial sympathectomy was attempted by injecting a little carbolic into the coats of the femoral artery.

Sepsis occurred, however, in the grafted area and evidently the increased blood flow swept emboli to the lung, the patient dying of pyaemic abscesses.

The results of calcium chloride intravenously have been disappointing. Where there are subcutaneous extensions, it forms abscesses ; it causes a sloughing of the skin in toxaemic patients if a drop leaks out from the vein ; veins thrombose ; and children—who need such a thing most—cannot have it. I have had three cases develop new ulcers while actually under treatment with it, nor can these people lack calcium when they swallow so much lime with their betel-nut. CLUNIE in Fiji found no diminution of blood-calcium in his cases.

DISCUSSION.

Let us consider the tropical ulcer which starts *de novo*, as showing all the stages (see p. 652), and as occurring both to the east and to the west of the south Pacific.

The first sign of this ulcer both in Fiji and in Melanesia is an inflammatory vesicle, the ordinary skin cocci being found in the contents. From the evidence given above it appears that in Fiji these skin organisms in patients predisposed to ulceration by a dietetic deficiency, are able to produce a lesion, which if untreated increases in size while remaining a simple ulcer. In Melanesia (west) on the other hand, it becomes infected with phagaedenic organisms, which convert the simple ulcer into the terrible phagaedenic ulcers we see here. The more predisposed the patient is, e.g., in New Britain, by poor nutrition, the more active will be the process and the more damage done. When a whole community is thus predisposed, we find an epidemic occurring. This epidemic is assisted by an increased virulence of the organisms by passage through very susceptible people.

Treatment consists of (1) cut short the primary infection by a dab of iodine, or later carbolic or blue-stone ; (2) relieve the predisposition by ample food, and (3) treat any malaria, because of the startling correlation between the distribution of ulcers and this infection.

The dietetic deficiency is usually an insufficient intake, these people being vegetarians with a precarious food supply. On the other hand, a good diet may be neutralized by the malarial destructive processes.

The evidence produced suggests the essential lack to be protein.

From a public health point of view, the people should be taught the necessity of ample food, and of the value of meat and fish. Training in better methods of catching fish and encouragement in rearing fowls, pigs and cattle for food purposes (and not for sale !) would do more good than merely the diminishing of the ulcer rate.

SUMMARY.

1. The paper discusses 892 cases of ulcer admitted to three hospitals in three parts of the south-western Pacific (Melanesia).

2. Phagaedenic ulcer is due to an infection with fusiform bacilli, spirochaetes and filamentous organisms.

3. There is a striking correlation between the clinical state of the ulcers and the organisms found in smears. All ulcers in the phagaedenic state showed fusiform bacilli.

4. Fusiform bacilli are the first of the phagaedenic organisms to appear. Smears show a gradation from a typical spirochaete through various filamentous forms to a typical fusiform bacillus. The possibility that they represent one organism cannot be dismissed.

5. The finding that clean or septic ulcers do not show phagaedenic organisms is opposed to the statement that all ulcers in the tropics contain spirochaetes.

6. The sequence of events between a break in the skin or the " idiopathic " ulcer through the fully developed phagaedenic ulcer to the healed ulcer is shown.

7. A dietetic deficiency is an important predisposing cause.

8. In New Britain, ulcers and their complications were the chief cause of mortality in hospital. One death only occurred in the Solomons.

9. A relationship between phagaedenic ulcers and malaria is suggested.

10. The virulence of the organisms may be increased by transmission amongst susceptible people.

11. As a prophylactic measure, the application of iodine and adhesive to small abrasions, etc., has been successful.

12. Treatment is carried out by destroying the infection in small ulcers by means of caustics, and by excision in the case of large ulcers followed by skin grafting. Any concomitant malaria or dietary deficiency must be dealt with.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

ATTEMPTS TO DEMONSTRATE LEPTOSPIROSIS IN THE NORTHERN SUDAN.

BY

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Jaundice is a very frequent clinical condition in the Sudan. Most of the common pathological causes which give rise to jaundice elsewhere are regularly encountered. A notable exception is cholecystitis, which is an extreme rarity in the Sudanese according to both clinical and postmortem records. Leptospirosis is apparently another. Considerable attention has been given by various workers to the possibility of leptospiral infection in some of the more obscure types of jaundice, but the spirochaete of Weil's disease has never been found.

ATTEMPTS TO IDENTIFY LEPTOSPIRA IN CASES OF EPIDEMIC AND SPORADIC JAUNDICE.

WHITEHEAD (1922) investigated an epidemic of jaundice in Port Sudan in 1922. Coarse spirochaetes were found in the urine in two cases but they were avirulent on inoculation into guineapigs, and all the other findings were against a diagnosis of Weil's disease. During 1924 and 1925, epidemics in Kassala

*The writer would acknowledge his indebtedness to Dr. E. D. PRIDIE, Director, Sudan Medical Service, for permission to publish this work, to Dr. A. E. LORENZEN, Dr. W. F. TOWNSEND COLES, and other members of the Sudan Medical Service, for help in obtaining rats, and especially to Major H. C. BROWN, for carrying out the serological tests, and for his kind advice and criticism on all points.

and Malakal were studied by WHITEHEAD and CROUCH (1926). A further epidemic was investigated by DAVIS (1931), but no evidence of leptospiral infection was discovered, either bacteriologically or serologically.

In 1929, cases of sporadic jaundice in the Northern Sudan were studied with the definite object of revealing Weil's disease if it existed. But in no case was it possible to find leptospira. Cultures of the organisms were procured from England, and the adhesion test, which had previously been shown by BROWN and DAVIS (1927) to be specific in leptospirosis was carried out with the sera of jaundiced patients. The results were uniformly negative.

During 1934-1935, a further series of 130 cases of sporadic jaundice was investigated in Omdurman but no evidence of Weil's disease could be obtained either bacteriologically or serologically.

Every year comparable numbers of jaundiced patients are observed in all the other hospitals throughout the Sudan, but Weil's disease has not been reported.

JAUNDICE OF OBSCURE ORIGIN.

The exclusion of leptospirosis does not mean unfortunately that the etiology of the jaundice is clear in all cases. Particularly in the course of the yellow fever survey which has been carried out in the Sudan during the last 4 years, attention has been drawn to numbers of cases of a clinical syndrome of unknown origin. The symptoms are usually moderate fever, deep jaundice, slight albuminuria, and in some cases, vomiting. In some of the fatal cases a high polymorphonuclear leucocytosis has been noted. The disease has been observed to occur in small outbreaks, and a considerable number of the afflicted have died. Clinically the syndrome is liable to confusion with yellow fever, but mouse protection tests have always given negative results. In no case has leptospira been demonstrated, while agglutination tests carried out by Major H. C. BROWN, of the Wellcome Bureau of Scientific Research, London, have always been negative. On several occasions it has been possible to investigate small outbreaks more fully. All examinations of blood, faeces and urine have been negative, and the nature of the condition is at present quite obscure. Resemblances have been noted, especially in the histology of the liver lesions, between these cases and cases of an obscure infective disease associated with jaundice, described by BEEUWKES, WALCOTT, KUMM, and HUDSON (1931) in Nigeria.

ABSENCE OF LEPTOSPIRAL INFECTION IN RATS.

The spread of Weil's disease is almost entirely due to the fact that rats (and other small rodents) harbour the organism, and excrete it in the urine. Surveys have revealed the presence of virulent leptospira in rats in almost all parts of the world. Even in India, where the evidence of human leptospirosis is so

scanty, TAYLOR (1937) found leptospira in 33 out of 100 *Rattus norvegicus* in Bombay. I have been unable to find any records from Arabia or Persia, but in Egypt SANDIFORD (1937) examined a series of twenty-three rats and failed to find any evidence of leptospiral infection.

A survey of the rats of the Northern Sudan has given similar negative results. A total of 259 rats have been examined by the following methods.

(1) Dark ground illumination of the kidney tissue (90 rats).

(2) Inoculation into guineapigs of saline emulsions of kidney tissue (105 rats).

(3) Agglutination tests, carried out by Major H. C. BROWN, against the London human strain of *L. icterohaemorrhagiae* which is serologically identical with the London rat strain of this organism (164 rats).

All those examinations have consistently failed to show any evidence of leptospiral infection. The rats were obtained from Khartoum, Khartoum North, Omdurman, Wad Medani and Port Sudan at various times of the year and the species distribution is as follows :—

Rattus rattus, 55 *per cent.*, *R. norvegicus*, 42 *per cent.*, *Arvicanthus testicularis*, 3 *per cent.*

DISCUSSION.

It is evident that these observations indicate the entire absence or extreme rarity of Weil's disease in the Northern Sudan, but whether some of the obscure cases of jaundice noted represent leptospirosis in a hitherto unrecognized and aberrant form cannot be stated definitely. MANSON-BAHR (1935) states that leptospirosis as it occurs in the tropics is said to be more virulent than it is in Europe, but that this point requires further investigation as also does its distribution in hot countries. The case described by DAS GUPTA and CHOPRA (1937) suggests that in the tropics there may be extreme difficulty in finding the organism. The negative agglutination tests in the Sudan cases indicate at least that if the jaundice had an undiscovered leptospiral etiology the organisms concerned must be serologically different from the classical strains.

The absence of rodent infections, too, is noteworthy. Rats are common in most of the towns of the Northern Sudan, but the arid desert environment is not a congenial one for an extra-corporeal survival of the leptospira.

ALSTON and BROWN (1937) point out that although leptospirosis is practically world-wide in its distribution, there is an apparent absence of the condition in Egypt, Arabia and Persia, as knowledge exists at present. These authors remark that the strong sunlight in these lands may be an important factor, for it is well known that bright light has a highly lethal action on leptospira.

It is possible that in the Northern Sudan, where climatic conditions are comparable to those of Egypt, Arabia and Persia, the position with regard to leptospirosis may be similar.

SUMMARY.

(1) The prevalence of jaundice in the Sudan is commented upon, and the occurrence of an apparently infective type of jaundice of unknown etiology is noted.

(2) Various observers have consistently failed in attempts to identify Weil's disease.

(3) Rats from the Northern Sudan have been examined for evidence of leptospiral infection, with negative result.

(4) Certain climatic features of the Northern Sudan, unfavourable to the spread of Weil's disease, are noted.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

A NOTE ON SOME CASES OF DISSEMINATED SCLEROSIS.

BY

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During the past year a number of cases were seen which correspond in in all essential signs and symptoms with disseminated sclerosis. That this disease occurs in the Gold Coast has long been known but it is doubtful if, hitherto, its frequency of occurrence has been marked as in recent years. It seems to correspond in that respect with the greater frequency of the disease in England where to-day it is far more common than the parasyphilitic neuropathic conditions which 20 years ago held the field.

A group of cases, eleven in number—on which this note is based—has been studied from certain angles and the signs and symptoms in each case have been classified in the accompanying table.

In the three last cases, which in point of time were studied first, certain signs were omitted from the notes and a dot, therefore, indicates "no observation."

It will be noted that fever was a fairly constant symptom but not necessarily of bad prognosis. In one case, the temperature rose to 105° F. day after day, yet the patient made a complete recovery.

Symptom. Name and age.	E. E. P., 28.	C. A., 19.	A. A., 30.	A. P., 15.	N., 24.	A., 28.	K. M., 25.	A., 18.	S., 30.	K. Y., 30.	A. A., 25.
Weakness or *paresis* of lower limbs	+	+	+	+	⊕	+	+	+	⊕	⊕	⊕
Altered *speech*—slow or monotonous	⊕	+	+	⊕	+	+	+	+	+	+	+
Knee jerks absent or exaggerated	abs.	abs.	abs.					abs.			
	+	+	+	+	+	+	+	+	+	+	+
Delusions	—	⊕	—	—	+	—	—	+	—	—	+
Fever. 100°F. or more	—	+	+	—	—	+	+	+	+	+	+
Defective vision, pale temporal half	+	+	+	+	+	⊕	⊕	+	+	.	+
Retention with overflow	+	—	⊕	—	—	—	—	+	.	.	+
Nystagmus	—	—	—	—	+	+	—	+	.	.	.
Epileptiform attacks	—	—	—	—	+	—	—	+	.	.	.
Intention tremor	+	+	+	—	+	—	+	..	.	.	.
Result—Death, Recovery ...	D.	R.	D.	R.	D.	R.	R.	D.	R.	R.	D.

N.B.—A circle indicates the symptom for which the patient or his friends sought treatment. A dot indicates that no note was made of the sign or symptom.

Nystagmus was only observed in three cases. In only one case was it marked.

In every case in which an examination was made the temporal half of the optic disc was found to be markedly pale and this condition cleared up as the disease passed off.

It will be observed that the ages varied between 15 and 30 years.

In this series there were five deaths. One from epileptiform convulsions which persisted till coma set in. One from an ascending paralysis starting with the bladder. One from mental aberration and two from general weakness with heart failure and signs of mental disturbance.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

SCHISTOSOMIASIS IN THE KAVIRONDO DISTRICT OF KENYA COLONY.*

BY

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I.—VECTORS OF *Schistosoma haematobium.*

Various methods have been used for deciding which species of snail is actually responsible for the development of the cercarial stage. Consequently in giving

*This paper is published by permission of the Hon. the Director of Medical Services, Kenya. The work was embodied in a thesis for the M.D. degree.

The writer's thanks are due to Major CONOLLY and Professor LEIPER for the identification of snails and schistosomes; to Mr. LEWIS of the Medical Department, Mr. HAMMOND and Mr. JOLLEY of the Veterinary Department, for their help and information; Mr. BENSTEAD for the timely supply of experimental rabbits and to Dr. VINT for sections of the bladder polyp, and his opinion on them.

a list of molluscs reported as intermediate hosts the method used will be indicated by the appropriate letter: (a), (b), (c), (d), (e), (f), after the author's name.

(a) Presence of a particular species of snail in water believed to be a source of infection or in waters situated in a district with a heavy human infection.

(b) Presence of cercariae resembling those of *S. haematobium* in " wild " snails.

(c) Attraction by a species of snail for miracidia.

(d) Recovery of adult schistosomes from the veins of an animal exposed to the cercariae obtained from naturally infected snails.

(e) Infection of " wild " snails with miracidia and the recovery of the adult worm from an animal exposed to the cercariae obtained from these snails.

(f) Infection of laboratory-bred snails with miracidia, the development of cercariae and the subsequent recovery of the adult schistosome from animals.

The conditions of (a) are fulfilled in (b), (c), (d), (e) and (f), and of (b) in (d) and (e).

Now the methods (a) and (c) are clearly not conclusive proof that the snail in question acts in that locality as the intermediate host. The experiment (b) is sound only if the cercariae can be identified with certainty: experiment (d) however is conclusive provided that all chance of cercariae being carried over in the original water is eliminated and that there is no possible doubt as to the identity of the adult schistosomes. Method (c) is an improvement on (d), while (f) when carefully carried out is the ideal final experiment.

A possible error may be present even when methods (e) and (f) are used, that is, that under experimental conditions infection by miracidia may be induced in a snail species which under natural conditions may not be the main carrier of infection.

The unsoundness of method (b) which has been pointed out by BLACKLOCK and THOMPSON (1924a), LANE (1936), and others is clearly shown by the disagreement of authors as to the true morphology of the cercaria and the similarity between those of *S. mansoni* and *S. haematobium*, which has been demonstrated by GORDON, DAVEY and PEASTON (1934) who " failed to reveal any points of difference " and between these and that of *S. bovis* pointed out by BRUMPT (1930), who says " Les cercaires de *S. bovis* ressemblent beaucoup à celles de *S. haematobium* et *S. mansoni*," and does not produce any unmistakable distinctions. A further illustration of the difficulties of method (b) shows some irony, for FAUST (1919) quoting CORT (1919), says with good reason of CAWSTON that " his descriptions and figures of this and other forked-tailed cercariae which he has described are so entirely inadequate that it seems to me that his entire work needs verification by more competent observers." At the same time FAUST identified both *S. mansoni* and *S. haematobium* cercariae from a single snail supplied by CAWSTON, who expressed some doubt as to the correctness of this last observation. In 1926 FAUST (1926) had to admit that he had been mistaken in the number of cephalic glands in the cercaria of *S. haematobium*.

Even the adult *Schistosoma bovis* can be confused with *S. haematobium* unless a careful examination is made, as reference to the table on page 683 will show: for apart perhaps from the extent of the vitelline glands, the shape of the ova is the only very distinctive difference. Therefore even the recovery of a few male or immature adults may be inadequate for differentiation. VAN DEN BERGHE (1936) has commented on this difficulty in a paper discussing pleomorphism in *S. haematobium* ova.

Molluscan Host.	Reported by	Method	Locality.
Bulinus contortus (Michaud)	LEIPER (1915-1918)	(c) (d)	Egypt
	CAROSSE (1930)	(b)	Southern Morocco
" *dybowskii* (Fischer)	ANDERSON (1922, 1923)	(a)	Tunis
	LEIPER (1915-1918)	(c) (d)	Egypt
" *innesi* (Pallary)*	ANDERSON (1922, 1923)	(a)	Tunis
" *truncatus* (Audouin)	LEIPER (1915-1918)	(c) (d)	Egypt
	MILLS, MACHATTIE & CHADWICK (1936)	(d) (e)	Iraq
" *brocchii* (Ehrenberg)	ANDERSON, (1922, 1923)	(a)	Tunis
" *forskalii* (Ehrenberg)	CAWSTON (1923)	(b)	Natal
" " "	ADAMS (1934, 1935)	(c) (d) (e)	Mauritius
Physopsis africana Krauss	CAWSTON (1922)	(b)	Lourenço
" " "	PORTER (1920)	(d)	South Africa
" " "	BECKER (1916, 1917)	(b) (d)	Transvaal
" *globosa* (Morelet)	BLACKIE (1932)	Male adults only (d)	Southern Rhodesia
" " "	BLACKLOCK & THOMPSON (1924)	No description of adults (d) (e)	Sierra Leone
" " "	GORDON, DAVEY & PEASTON (1934)	Male adults only (d) (f)	"
" *nasuta* von Martens	CORSON (1925)	(b)	Tanganyika
" *ovoidea* Bourguignat	CAWSTON (1927)	(a)	Zanzibar
Limnaea natalensis Krauss	PORTER (1920)	(d)	South Africa
Planorbis metidgensis Forbes (or *P. dufourii* Graells)	BETTENCOURT & BORGES (1922)	(d)	Portugal
Melania (*Melanoides*) *nodicincta* Dohrn	DYE (1924)	(a) (c) And recovery of furcocercariae from artificially-infected wild *Melania* (b)	Nyasaland
Melania (*Melanoides*) *tuberculata* (Müller)	GOPSILL (1930)		Nyasaland

*PALLARY himself states BOURGUIGNAT to be the author, but incorrectly.

II.—Bilharzia in Kenya.

In this colony both the urinary and intestinal forms are widespread, but, as elsewhere, they occur in certain limited areas. Few references to the presence of the urinary form of schistosomiasis are available, except in routine reports, though its presence in Central Kavirondo was mentioned by Ross (1929) and in Nyanza Province by Milton (1922). This form is found in Nairobi, Mombasa, Malindi, Kilifi, Digo, Teita (Mwatati area according to Dr. P. C. C. Garnham), Machakos, Kitui, Kisumu, Kisii and Kakamega. It would appear that the most important areas are the coastal belt and round the shores of Lake Victoria; in other places the sources of infection are comparatively small localized districts.

The toxins produced by *S. haematobium* appear to be far milder than those of *S. mansoni*, and do not give rise to severe involvement of the liver and spleen (Girges, 1934), while attendance at hospital for complications in the urinary system has not been reported.

As far as the writer is aware there has been no investigation in this colony as to the intermediate host of *S. haematobium*, although a fruitless search was once made at Mombasa (Cawston, 1928).

III.—Urinary Schistosomiasis in Central Kavirondo.

(1) topography of district.

Central Kavirondo is a district lying to the north of the Kavirondo Gulf of Lake Victoria and urinary schistosomiasis is strikingly confined to a clearly limited and small strip of country roughly 20 miles long by 4 miles deep lying along the northern shore of the gulf. This area, rising gradually from the water level of 3,700 feet by a few hundred feet, is mostly open country, agricultural and pastoral, with some small rocky hills. There are several streams running from the higher ground towards the lake, but these are mostly a sequence of pools either grassy or rocky with sometimes a stream running between them, according to the rainfall. Most of these pools are used for watering cattle, bathing and as a supply for all domestic purposes by the natives.

This distribution of the disease refers only to the district of Central Kavirondo and it probably exists in areas along the neighbourhood of the lake shore right round to the east and south.

The rainfall is probably very similar to that of Maseno situated about 10 miles from the eastern end of this strip. At Maseno the annual rainfall averages 54 inches, with shade temperatures ranging from a maximum of about 90° F. to a minimum at night of 55° F. The relative humidity at Kisumu on the lake shore ranges from 50 to 75 per cent. There is not a very marked seasonal variation in temperature. The area with schistosomiasis, however, is several

hundred feet lower in altitude than Maseno, and would appear to be warmer with probably a humidity much the same as at Kisumu.

(2) INCIDENCE OF INFECTION.

As records of schistosomiasis at hospitals and dispensaries are of little value for the estimation of the incidence of this disease, except as an indication of its presence or absence, all the children from 3 to 12 years of age, from one "mlango" or group of villages under one headman were collected and their urines examined for ova. The following results were obtained :—

	Number Examined.	Number with Ova in Urine.	Number with Urine with Red Blood Corpuscles but no Ova.	Number with a history of Infection but Urine Clear.	No history of Infection and Urine Clear.
Male	97	74	11	1	11
Female	26	14	6	—	6
Total	123	88	17	1	17

As histories are unreliable, the one case with a positive history but clear urine will be considered as a negative ; while experience indicated that in all cases showing red blood corpuscles in the urine but no ova, further examination would reveal ova. If these cases are counted as positive, it appears that 85 per cent. of the children are infected. There is also some indication that the rate for girls is not so high as that for boys ; this might be expected, as only the boys herd cattle and are more constantly exposed to infection by standing and wading in pools, when the cattle are taken to water.

(3) MEASUREMENTS OF OVA.

The ova in the urine were typical of *S. haematobium*, healthy specimens measuring from 114μ to 164μ in length, and from 53μ to 68μ in breadth, with an average size of 139μ by 59μ. A small number of ova obtained from youths aged about 19, declaring themselves to be free from infection, gave average measurements of 107μ by 49μ ; not a sufficient number, however, were examined to put forward a suggestion that the immunity developed by this age could be

influential in the production of small-sized eggs. All ova were measured immediately after being passed, lying in the urine without a coverslip.

Average measurements given by BRUMPT (1927), GIRGES (1934), ARCHIBALD and MARSHALL (1932) and GORDON, DAVEY and PEASTON (1934) are 150μ by 60μ, 146μ by 56·1μ, 140μ by 60μ, and 143μ by 60μ respectively.

No ova resembling those of *S. bovis* (MACHATTIE, MILLS and CHADWICK, 1933) or *S. matthei* (BLACKIE, 1932) were seen.

(4) THE FRESHWATER MOLLUSCS.

A search of the pools and streams produced four different types of snails (see Plate) :—

(i) *Limnaea* [*natalensis* Krauss ?].
(ii) *Planorbis stanleyi* Smith.
(iii) *Bulinus* (*Pyrgophysa*) *forskalii* (Ehrenberg).
(iv) *Physopsis nasuta* von Martens.

Major M. CONOLLY, of the British Museum very kindly identified these snails.

Both *Limnaea* and *Planorbis* were scanty, although the former was present in considerable numbers a few miles away where schistosomiasis did not appear to be present.

The last named species (iv), however, included what appear to be two varieties (see Plate), but as Major CONOLLY points out there may be considerable variation in any species.

Oecology.—The pools in which *P. nasuta* were found form a series connected by streams after heavy rain, but more often separated by damp ground only. Infected snails were recovered chiefly from those pools lying in a gully and shaded in the early morning and evening. The temperature of the water was taken a few inches below the surface and in the shade ; the following readings were recorded : Maximum, 28·6° C. ; Minimum, 17·2° C.

Physopsis as well as *Planorbis* were generally found to be on the grass overhanging the edge of the pools, just below the surface of the water, and also among the dead leaves lying on the muddy bottom just below water level. All the species of snails do not seem to be present together in the same pool : *Planorbis* was found associated with *B. forskalii* in one place and with the shorter spired variety of *P. nasuta* in another.

B. forskalii was much more scarce than the other species, was associated with clearer and less stagnant water than the others, usually in slowly flowing water, and appeared both to get easily swept away by a rapid flow, and on the other hand to die off when a pool became stagnant.

It was also noticed that although *P. nasuta* could be kept easily under laboratory conditions by the daily addition of a little fresh rain water, *B. forskalii* could never be induced to survive for more than 8 days, and the latter when

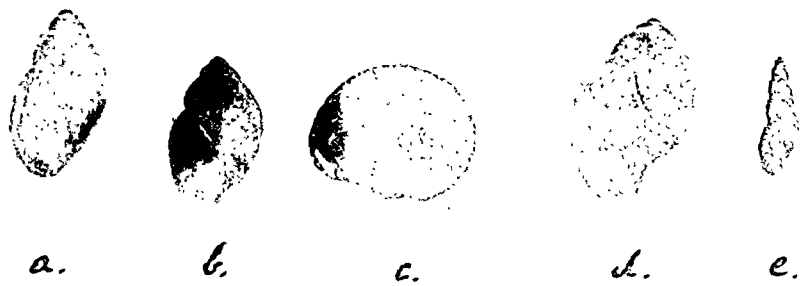


FIG. I

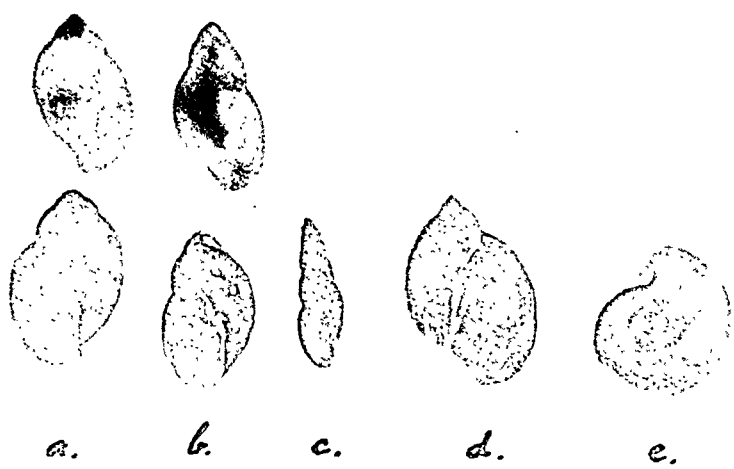


FIG. II

FIG. I.—(*a*) *Physopsis nasuta* von Martens, variety 2.
(*b*) *Physopsis nasuta* von Martens, variety 2.
(*c*) *Planorbis stanleyi* Smith.
(*d*) *Physopsis nasuta* von Martens, variety 1.
(*e*) *Bulinus* (*Pyrgophysa*) *forskalii* (Ehrenberg).

FIG. II.—(*a*) *Physopsis nasuta* von Martens, variety 1.
(*b*) *Physopsis nasuta* von Martens, variety 2.
(*c*) *Bulinus* (*Pyrgophysa*) *forsakalii* (Ehrenberg).
(*d*) *Limnaea* [*natalensis* Krauss ?]
(*e*) *Planorbis stanleyi* Smith.

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kept for transport in moist sand or grass showed a much greater mortality than the former.

At Maseno, which is several hundred feet higher in altitude, there is no schistosomiasis, which indicates that the conditions are either unsuitable for the development of the intermediate host, the liberation and survival of miracidia (BOUET and ROUBAUD, 1912) or the development of the asexual cycle within the snail, as infected natives are undoubtedly present. The temperatures of pools at Maseno taken under similar conditions to the above gave a variation of from 20° C. to 23·9° C., which does not suggest that the temperature is a decisive factor.

According to GORDON, DAVEY and PEASTON (1934), the optimum temperature for the development of cercariae in *P. globosa* was 27° C. to 33° C., though this species could tolerate higher temperatures than *P. pfeifferi*, while the temperature in pools harbouring *P. globosa* varied between 20° C. and 30° C. They conclude that " it is highly unlikely that in Sierra Leone, water temperature plays any important part in limiting the distribution of this species." The influence of temperature on the development of the parasite in the snail has been suggested by BETTENCOURT and BORGES (1922) in Tavira, Portugal, where a pond transmitting infection had a temperature of 25·4° C. ISOBE showed in 1933 the influence of temperature on the escape of cercariae from the snail; while CONOR (1910) observed that bilharziasis was acquired in springs with a temperature of 28° C. but not in those at 50° C.

It appears that the factors controlling the successful evolution of the schistosome cycle in the snail in nature are not sufficiently understood, whether the flora, the food supply of the host, the soil or the chemical or physical conditions of the water have an important bearing on the problem.

A careful study of the oecology of the molluscan host would help to elucidate these factors.

(5) SNAIL DISSECTION.

Specimens of the above snails were collected and dissected with the following results :—

	Number Dissected.	Number with Furco-cercariae.	Number with Single-tailed Cercariae.	Number with Sporocysts only.	Number with no Sporocysts or Cercariae.
B. forskalii	140	0	6	7	127
L. natalensis	49	0	4	10	35
P. stanleyi	75	0	22	9	44
P. nasuta (type 1)	40	17	3	0	20
P. nasuta (type 2)	54	11	0	1	42

Most of these snails were collected from well frequented bathing and watering places.

(6) CERCARIAE.

The furcocercariae from the livers of *Physopsis* had the following characteristics :—

1. Bifid tail. 2. No pharyngeal bulb. 3. No pigment spots. 4. Two pairs of anterior and three pairs of posterior cephalic glands. 5. It was not found possible to determine the number of flame cells with certainty, but three or four pairs appeared to be present in the body, besides the pair in the tail.

Measurements were made :—

(*a*) *When living and in normal saline without a coverslip.*—Length of body while stretching 225μ, at rest 120μ. Breadth of body while stretching 26μ, at rest 54μ. Length of tail 150μ, length of furca 80μ.

(*b*) *Just dying in normal saline without coverslip.*—Length of body 150μ, breadth 54μ; length of tail 182μ, length of furca 82μ.

(*c*) *Under coverslip in saline, as evaporation caused pressure.*—Length of body 254μ, length of tail 291μ, length of furca 109μ.

(*d*) *After fixation in lactophenol.*—Length of body 159μ, breadth 55μ; length of tail 192μ, length of furca 60μ.

In order to compare measurements with those of other workers it is clearly necessary to use a carefully standardized technique.

The methods employed were those described by GORDON, DAVEY and PEASTON (1934): vital staining with alizarin for the anterior glands, brilliant cresyl blue for the excretory system, and lithium carmine for the posterior glands. The results obtained were as quoted above and indicated that the cercariae were morphologically similar to those of *S. haematobium* and *mansoni*, these authors stating that no clear distinctions can be made between the two species.

The unsatisfactory description of cercariae already referred to, can be appreciated when we see that FAUST (1919, 1920), BETTENCOURT and DA SILVA (1925), BETTENCOURT and BORGES (1922), CAWSTON (1922), ARCHIBALD and MARSHALL (1932), PORTER (1920), and MANSON-BAHR and FAIRLEY (1920), all describe three pairs only of " mucin," " head," " penetration " or " cephalic " glands, while FAUST in a later publication (1926), BLACKLOCK and THOMPSON (1924*b*), MOHAMED (1931, 1932), and GORDON, DAVEY and PEASTON (1934) report the presence of five pairs. There is also disagreement over the number of flame cells.

(7) ANIMAL EXPERIMENTS.

It was decided to attempt to identify the species of cercariae found, by infecting animals. Fortunately some rabbits were available. These were

infected either by shaving part of the ear and holding it in water containing cercariae from the naturally infected snails or by placing a few drops of water with cercariae on a shaven portion of the skin for 20 to 30 minutes.

Rabbit.	Time between Infection and Dissection.	Result of Autopsy.
1	Five months after exposure to water at 21° C., containing infected snails	Negative
2	Seven weeks after exposure to water at 21° C., containing infected snails	"
3	Two months after exposure to cercariae from liver of snail	"
4	Three months after exposure to cercariae from liver of snail	2 males, 2 females 2 immature males
5	Three months after exposure to cercariae from liver of snail	Negative

Monkeys, rats, mice, dogs, rabbits, etc., have been used, but FAIRLEY, *et al.* (1930) suggest that certain animals, including guineapigs, may not be propitious hosts, owing to such results as the finding of immature males only or the non-production of eggs, an opinion endorsed by INGRAM (1924), GORDON, DAVEY and PEASTON (1934) and by MILLS, MACHATTIE and CHADWICK (1936) with guineapigs.

In Rabbit 4, two pairs *in copula* and two immature males were recovered from the liver or portal vein. These were stained with hydrochloric carmine according to the method described by GIRGES (1934), and were identified by Professor LEIPER as *S. bovis*, one female having in its uterus typical *S. bovis* ova, while the other had several eggs with *S. haematobium* outlines, and one typical *S. bovis* egg.

A second animal experiment was carried out when the writer was transferred to Nairobi. Snails were transported to Nairobi, where furcocercariae obtained from *P. nasuta* (type 1) were used to infect white mice. The mice were exposed by standing them in a little water containing the cercariae, for half an hour.

Mouse 1.—Exposed to over 100 cercariae—killed 60 days after infection, produced fifteen females and eight males (some immature) and showed a tumour-like mass in the bladder as well as an extensively diseased liver.

Mouse 2.—Exposed to about fifty cercariae—killed 61 days after infection, no schistosomes found. No gross disease of organs was seen, but a papillomata-like mass was present in the bladder.

Mouse 3.—Exposed to about sixteen cercariae—killed 61 days after infection, no schistosomes found.

All the adult schistosomes recovered were identified as *S. bovis*, the females having typical ova in their uteri (see Text Fig. 1, p. 682).

It was noticed that in one stained preparation, the ventral sucker of the female was prominent and more or less pedunculated (see Plate Fig. 2), whereas KHALIL (1924) describes the ventral sucker as " hardly visible in the majority

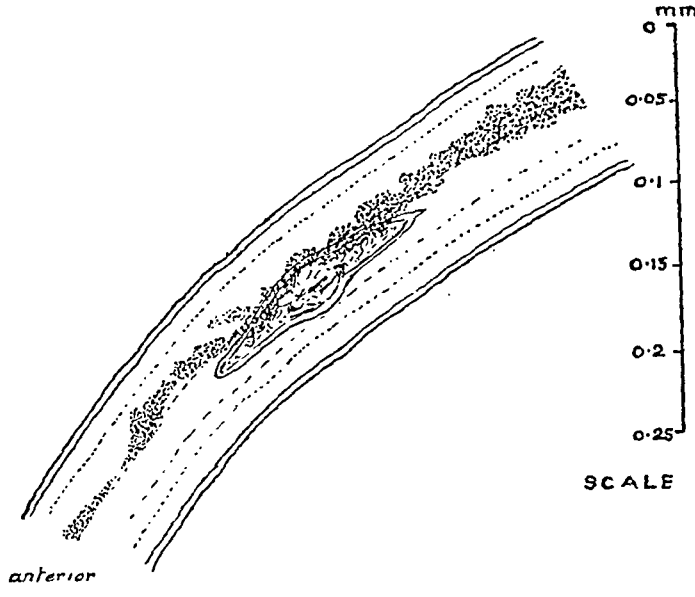
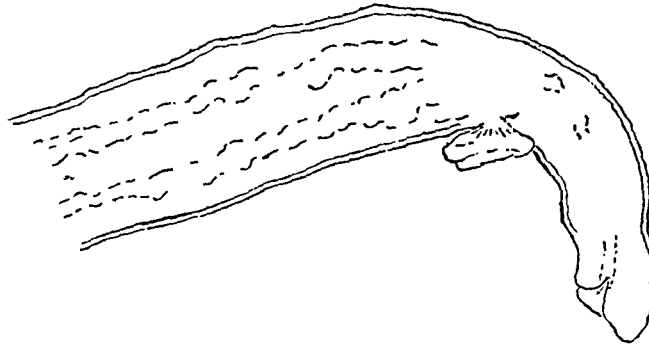


FIG. 1.—Ovum in the uterus.



(*Camera lucida drawings.*)

FIG. 2.—Showing ventral sucker (female).

of cases and does not project above the surface of the worm." In other respects, these adults conformed to the description of KHALIL.

Sections of the mass in the bladder show what appears to be a worm, which may indicate that *S. bovis* is capable of affecting the genito-urinary system (BRUMPT, 1930; MACHATTIE, MILLS and CHADWICK, 1933).

IV.—*Schistosoma bovis.*

This parasite of sheep, cattle, horses and donkeys was first described by SONSINO in 1876. It has a fairly wide distribution, being found in Egypt,

	S. bovis, As described by KHALIL (1924).	*S. haematobium* As described by R. GIRGES (1934).	*S. matthei* As described by VEGLIA and LE ROUX (1929).
Ova			
Measurements	160–180μ by 50–60μ 130–260μ by 40–95μ (MACHATTIE & CHADWICK)	146·4μ by 56·1μ	170–280μ by 72–84μ
Adult Male			
Measurements	9–14 mm. Spindle shaped anterior portion not folded, posterior folded	11–12 mm. by 1 mm. Anterior portion resembles truncated cone	17–22 mm. by 1 mm.
Testes	Four (3 to 6)	Four or five	Four to Six
Oral Sucker	0·23 mm. broad, 0·15 mm. deep, funnel shaped, very obliquely placed	0·4 mm. in diameter obliquely placed	
Ventral Sucker	0·42 mm. in diameter, not pedunculated, covered with spiny processes and lined with small spines	0·4 mm. or a little larger than oral sucker. Short, thick peduncle	
Pharynx	None	None	None
Oesophagus	0·5 mm.	0·4 mm.	
Caeca	Two starting just above V. sucker, unite near beginning of posterior quarter of worm. Occasionally divides and unites two or three times	Two starting just above V. sucker, unite in posterior half of body. Final caecum may divide and unite again	Two caeca may fail to unite, or may unite close to posterior extremity. Bifurcation may recur two or three times
Cuticle	Tubercles with small spines on dorsal aspect. Anterior portion of worm smooth	Dorsal aspect posterior to V. sucker, covered with wart-like papillae tipped with bristles. Ventral surface also invested with spinous papillae	Anterior portion smooth. Dorsum with spine bearing cuticular tubercles. Ventral surface with spines
Adult Female			
Measurements	12–17 mm. cylindrical	About 20 mm. cylindrical	17–25 mm.
Oral Sucker	0·04 mm. broad, not so definite as in male	0·07 mm.	Suckers covered with spines, but smaller than those of male
Ventral Sucker	Hardly visible, does not project above surface of worm, 0·05 mm.	0·059 mm. pedunculated	
Caeca	As in male, but does not branch again	As in male	Reunite in posterior half of worm, terminate within 0·1 of end
Ovary	0·3 mm. by 0·15 mm. pear-shaped just in front of junction of caeca	In older posterior end club-shaped, attenuated anteriorly	1 mm. by 0·2 mm.
Vitelline Glands	Start 0·1 mm. posterior to ovary, fill all posterior part of worm to 0·2 mm. from end	Occupy the posterior fourth of the body	Occupy practically whole posterior half of worm
Cuticle	Smooth	Smooth except on suckers and towards posterior end	Smooth

Sicily (GRASSI and ROVELLI, 1888), India (BOMFORD, 1886), Malay (MACHATTIE and CHADWICK, 1932), South Africa, Mauritius, etc.

Its morphology may most concisely be indicated by a com *S. matthei* Veglia and Le Roux and *S. haematobium*, as in the table identity of *S. bovis* with *S. matthei* has been maintained by MA CHADWICK (1932, 1933).

Intermediate hosts of *S. bovis* are *Bulinus truncatus* in Iraq, and CHADWICK, 1932); *Physopsis africana* in Natal, (CAWSTON, 19 *contortus* in Corsica (BRUMPT, 1930); *Physopsis globosa* i (BLACKIE, 1932).

V.—DISCUSSION.

There seems, therefore, no doubt that *P. nasuta* is the inter of *S. bovis* in this district. It also appears likely that *S. haematob* mitted by either *P. nasuta* or *B. forskalii* or both. It has been s snail host of these two schistosomes is identical in at least two pla South Africa; so that it would not be surprising if a similar condition in Kavirondo. CORSON (1927) has suspected *P. nasuta* in Tangany on inadequate evidence. On the other hand, *B. forskalii* has been vector in Mauritius. Presuming that PORTER was correct in he that *L. natalensis* is a vector, it would appear that the same speci country may not be liable to infection, for other workers have fa this snail with miracidia, and all observers including BETTENCOURT have failed with *Planorbis* species. DYE (1924) failed to infec *L. natalensis* or *B. forskalii* with *S. haematobium* miracidia, VOGE failing with the first species, while INGRAM (1924) failed to infect *P. waterloti*, but succeeded with *Physopsis globosa*. The only di shown here is that in a district with an extremely heavy infection rat has a scantier distribution compared with *P. nasuta* and that of the 1 dissected none have shown furcocercariae, whereas of the cercaria in *P. nasuta* it is possible that some were of the human type, alth in the transmission experiments.

There has been no suggestion from routine examination of fae that other schistosomes than *S. mansoni* or *S. haematobium* may be as the types reported by BLACKIE (1932), FISHER (1934), WALL BRUMPT (1931), LE ROUX (1933) or CAWSTON (1925).

No animal schistosomiasis has been reported in Central Ka in the whole of Kenya only two cases, occurring in bullocks in the ne of Nairobi, have been seen by Mr. R. DAUBNEY, of the Veterin Laboratory.

What is possibly the development of an immunity [illegible]

judging from hospital reports, complications in the urinary system must be rare. To quote a single instance : The boy, aged about 18, who collected most of the snails examined, had had haematuria as a child and although he must have been exposed to repeated infection, he denied having any symptoms and an examination of his urine 5 months after beginning the work showed no ova or blood.

The days of prophylaxis, as represented on walls of Egyptian temples, by the use of the penis shield (PFISTER, 1913), by "the European equivalent of this ancient speciality" (LEIPER, 1915) or by circumcision (MADDEN) are perhaps past and we can look to more modern methods. Treatment alone can be of little value in a native population for the eradication of schistosomiasis, owing to continual reinfection. As in so many other tropical diseases, prevention is the better method, and as in all disease the ideal at which to aim. If the definitive host is only *Homo sapiens*, clearly restraint of all members of the community from urinating in the vicinity of water would very soon eradicate the disease ; but restraint and change of habits are not prominent characteristics especially among the natives of Central Kavirondo, so that it is necessary to resort to other measures ; although the writer considers that strenuous efforts to explain and demonstrate the methods of infection in conjunction with other means would contribute to final success.

Such methods as those advocated by LEIPER (1915*b*) for irrigation canals in Egypt do not apply here, but the use of species of *Tephrosis* (CAWSTON, 1928*b*) and the fruit of *Balanites* (ARCHIBALD, 1933) and of chemicals such as ammonium sulphate (LEIPER, 1915*b*), copper sulphate (CHANDLER, 1920 ; KHALIL, 1927), etc., might be tried in stagnant pools. The heavy rainfall, without much intermission, however, renders the maintenance of an adequate concentration of chemicals difficult. Such measures as the building of cement bathing pools, cattle troughs and washing places, although involving some expenditure, would be of more general value as public health measures and would probably be more readily accepted by the natives and consequently hold out the best hopes of control.

It is hoped that eventually further work may be done, both on the development of *S. haematobium* from naturally infected snails and on the more conclusive laboratory experiment by infecting bred snails with miracidia in order to establish definitely the rôle played by the species present in Central Kavirondo.

Cases of *S. mansoni* infection (intestinal) have been seen in another area of Central Kavirondo in natives, who state that they have never left the district and it seems likely that *P. stanleyi* Smith is the intermediate host here. A careful survey of the snail distribution in the Colony would be of great value in assisting to elucidate the factors involved in the distribution of the disease as well as the vectors concerned, and an investigation of animal schistosomiasis would also be of great interest.

VI.—Summary.

1. It is concluded that *Physopsis nasuta* is an intermediate host of *S. bovis* in Central Kavirondo.

2. The intermediate host of *S. haematobium* is probably *P. nasuta* or *B. forskalii* or possibly both.

3. It is fallacious to assume that the presence of furcocercariae of the "*S. haematobium* type" in snails proves that snail to be a vector of human schistosomiasis.

4. Further work is needed for the identification of the molluscan host in Kenya; a snail survey and an investigation of animal schistosomiasis would be of great interest.

5. Certain prophylactic measures might be instituted.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. XXXI. No. 6. April, 1938.

A CASE OF AMOEBIC HEPATIC ABSCESS AND ASSOCIATED PERICARDITIS.

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Sekondi, Gold Coast.

Amoebiasis is common in the Gold Coast. During 1937, four cases of amoebic hepatitis have been treated in Sekondi hospital. I record the following case, as I have not known of pericarditis in association with amoebiasis.

Case 3, *Issaka, F.*, male, aged 30 was admitted to hospital, 15.12.37, complaining of pain in right chest and hypochondrium for 4 days. He had suffered from diarrhoea for 2 weeks before admission. On examination the patient was obviously gravely ill, the breathing laboured. Temperature 99·6° F., pulse 136, respiration 36. There was no cough but deep breathing was very painful on the right side. There was great increase in liver dullness, which extended from the right nipple line to near the umbilicus, where the free edge was palpable. In the lungs the air entry was clear except in the right fifth interspace ; there was audible friction in fifth and sixth right spaces. The cardiac apex beat *could not be seen or felt.* It was audibly weak and *distant*, but there was no definite increase in cardiac area by percussion. Blood count showed 3½ million red cells, 21,600 white cells. Haemoglobin : 35 per cent. Stool : no parasites or cysts.

Early diagnosis and prompt treatment were required. The possible diagnoses were, in order : amoebic hepatitis, probably with abscess formation ; pneumonia with empyema ; subphrenic abscess ; and pericardial effusion or pyopericardium. The clear lungs, increased liver area, and the leucocytosis favoured amoebiasis. Emetine was administered ½ grain twice daily with slight but certain improvement which continued. But the liver reduced little in size, so abscess formation was more certain. The cardiac signs caused concern ; the absence of signs of apex thrust, its audible faintness and the weak pulse strongly suggested pericardial effusion ; the other explanations of these signs were a much thickened pericardium (unlikely), emphysema, or fluid in the left chest ; confirmatory signs were absent and the apex beat, although faintly audible was not displaced. After a week's emetine treatment these signs altered, the apex beat then being felt in its normal position, while it sounded louder. At the same time there became audible a loud to and fro friction rub

synchronous with the heart beat, audible over an area centred in the right interspace beside the sternum. The sounds and the timing of this rub were easily appreciated by members of the nursing staff.

On the 15th day, the patient's progress was interrupted by a flare up. There was marked dyspnoea, pallor and weakness, pulse very faint (I considered that the abscess might have increased suddenly).

On the 20th day, the liver was aspirated under evipan anaesthesia, 2 pints of pus were withdrawn. A second course of 10 grains of emetine was administered. Recovery was uneventful. The pericardial rub disappeared, the heart function became normal. The patient was discharged in sound health on the 31st day.

COMMENTS.

1. The precise nature of the pericarditis is uncertain. As the abscess condensed, its position, central in the liver, was apparent, therefore, under the heart; proximity of this inflammation may have caused some effusion which resulted in chafing pericardial surfaces as the liver subsided on emetine treatment. There can be no question of leakage from the abscess into the pericardial sac; such must be rapidly fatal.

2. The delay in aspirating the abscess may be criticized. Twelve years ago a case was admitted to Kumasi hospital with great liver enlargement. Forthwith I aspirated and withdrew some pints of pus; the patient died within the hour.

It is necessary to reduce toxaemia and restore strength before aspiration, which should be slow. I use a large needle or smallest bore cannula, otherwise decompression shock may prove fatal. Also, it should be recognized that liver puncture in a very sick man is a *considerable* trauma.

OBITUARY.

FÉLIX MESNIL.

1868—1938.

Professor FÉLIX MESNIL, who had been an Honorary Fellow of The Royal Society of Tropical Medicine and Hygiene since 1921, died unexpectedly on 15th February. He was a native of Normandy, having been born at Omonville-la-Petite, near Cherbourg on 12th December, 1868.

After completing his school education he entered the Ecole Normale Supérieure in 1887 and graduated in Natural Sciences in 1891. In the following year he took up an appointment at the Institut Pasteur. There for 2 years he was secretary to PASTEUR, and at the same time worked with ELIE METCHNIKOFF and prepared a thesis for the Doctorate of Natural Sciences, his subject being the resistance of the lower vertebrates to artificial microbic infections. At the Institut Pasteur he was successively assistant, head of a laboratory (1898) and finally in 1907 director of the newly created department of colonial microbiology, a post which he held till his death.

A student of ALFRED GIARD, MESNIL was by education a zoologist, but under the direction of METCHNIKOFF and thanks to the influence of ROUX and also to some extent to his collaboration with LAVERAN in various researches he became a protozoologist and microbiologist. It is thus easy to understand the broadness of his outlook and the variety of problems he investigated. On the purely zoological side, though he conducted important researches on the Annelids, the parasitic Crustacea, the Orthonectids and the Turbelarians, he was above all a protozoologist. Thus, his works on the Sporozoa and the Cnidosporidia are recognized as classics. He it was who discovered the process of schizogony in certain Gregarines. He demonstrated the susceptibility of the chimpanzee to the human malarial parasite *Plasmodium vivax*, and as long ago as 1889 clearly defined the affinities of the Haemosporidia, pointing out the close relationship of the Haematozoa of malaria and the Coccidia. To him is due the discovery of a sexual process in the Actinomyxidia. This takes place by anisogamic copulation, a cycle of development which was all the more difficult of demonstration in that it presented certain unique features. The germinal part develops outside the spore envelope through which it penetrates actively after a maturation process. To MESNIL's credit is due again our knowledge of a number of new parasites in the groups Haplosporidia, Siedleckia, Toxosporidium and the curious protists parasitic on Gregarines, the Metchnikovellidae.

The supremacy of MESNIL as a morphologist revealed itself in his solution of the difficult problem of the systematic position of the spirochaetes. In 1901 he recognized the bacterial nature of the supposed trypanosome of the crystalline style of oysters, and thus established the affinities of the spirilla and the spirochaetes.

The meeting of LAVERAN and MESNIL, a medical man and a biologist, was the starting point of a series of researches on trypanosomes which MESNIL continued with his many pupils. The general structure of trypanosomes was studied, and the various constituents of the flagellar apparatus were homólogized with their equivalents in other flagellates and the spermatazoa. Entirely new forms, such as *Endotrypanum* and the trypanoplasms were brought to light. A detailed study was made of *Trypanosoma lewisi* infections in the rat, as also the course of experimental *T. brucei* infections in these animals. From these studies the possibility of adaptation of trypanosomes to various species of animal was realized. The agglutination of trypanosomes, without loss of motility by normal and specific sera, was discovered, while the cross immunity test, the test of Laveran and Mesnil, was introduced as a method of identifying trypanosomes. The trypanocidal and protective action of human serum against various animal trypanosomes was discovered, and it was shown that this property was not shared by the zoologically related sera of primates and monkeys. These various serological reactions were applied to the study of *Trypanosoma rhodesiense*, the characters of which in these respects proved to be distinct from those of *T. gambiense*.

The above-mentioned serological investigations led to the study of variation in trypanosomes and the experimental production of serum-resistant races which maintained this character after long passage through animals. Finally, the subject of the treatment of trypanosomiasis was taken up, and, as all know, the fruitful collaboration with MAURICE NICOLLE, in the early days of chemotherapy, led to the study of benzedine dyes and the discovery of the activity of trypan blue. Certain laws bearing on the relationship of chemical constitution to therapeutic action were formulated. Thus, the importance of diazotized benzidine, the naphthalene nucleus and the sulphonic acid and amine groups were fully recognized. The value of atoxyl for the treatment of *Trypanosoma gambiense* infections was established.

The foregoing account of MESNIL's researches will have shown how well qualified he was to undertake, as he did, with LAVERAN, the publication of the first general account of a protozoal disease—the classical treatise on *Trypanosomes et Trypanosomiases* of which two French editions were issued in 1904 and 1912 and one English edition in 1907.

Though not a medical man MESNIL's share in the development of tropical medicine in France has been considerable. During the early days of tropical protistology his influence on the many pupils, who passed through his hands, was such that it can be said, with confidence, that he was one of those who



J. Nesmith

contributed most to the establishment of French colonial medicine on a truly scientific basis.

For many years, and up to the time of his death, MESNIL was a contributor to the *Bulletin de l'Institut Pasteur*, of which he was one of the founders. There he reviewed papers on protistology, and workers in this field in all parts of the world had become accustomed to look forward to these reviews as a reliable guide to the literature of their subject.

In his own country MESNIL's claims as a scientist of great distinction were duly recognized by his election to the Académie des Sciences, the Académie de Médecine and the Académie des Sciences coloniales. He was at one time President of the Société de Pathologie exotique, the sister society to our own. In 1920 he was a recipient of the Mary Kingsley Medal of the Liverpool School of Tropical Medicine.

MESNIL was devoted to science and to the Pasteur Institute, where he spent the whole of his scientific career. There for over 45 years he carried out his investigations, either alone or in collaboration with LAVERAN, MAURICE NICOLLE and others, or took part in the training of the many students destined chiefly to work in the tropical possessions of his own or other countries. To those who came in contact with him he remained a firm friend. He was always pleased and gratified when any old student returning to France or passing through its capital found time to visit him in his department at the Pasteur Institute, and he extended to all a welcome which was simple and sincere. In his death those who knew him will have lost a real friend, and those who knew him only by repute one whom they have been long accustomed to admire for his honesty of purpose and the clearness of his judgment as exemplified by his writings. He was a protozoologist of great distinction, and his loss is all the more keenly felt on account of the difficulty of replacement.

ANNOUNCEMENTS.

TRUSTEES OF THE SOCIETY.

At the Council held on 17th February, 1938, Sir ARTHUR BAGSHAWE and Professor WARRINGTON YORKE were, in accordance with Law 20, appointed Trustees in place of the late Major AUSTEN and Sir THOMAS STANTON.

The three Trustees of the Royal Society of Tropical Medicine and Hygiene are therefore now—Dr. G. CARMICHAEL LOW, Sir ARTHUR BAGSHAWE and Professor WARRINGTON YORKE.

MANSON MEDAL.—1938 AWARD.

At the Council held on 17th March, 1938, the MANSON MEDAL was awarded to Major-General Sir LEONARD ROGERS, *K.C.S.I.*, F.R.S., I.M.S. (ret.).

The Medal will be presented at the Annual General Meeting to be held at Manson House, on Thursday, 16th June, 1938.